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CORRIGENDA

Volume 151, page 30, lines 4 and 6. The dissociation constant should read 2×10^{-6} instead of 2×10^{-3}.

Volume 152, page 340. Under table 2, top, right-hand column, "coronary, mean $\pm \sigma_m$", the first entry should read: $13.0 \pm .54$ instead of 13.0 ± 5.4.

Volume 154, page 37. Insert the following paragraph before SUMMARY AND CONCLUSIONS:

Finally, in connection with a discussion of T-1824 clearance, it is interesting and informative to note the tabulated data of electrophoretic studies on T-1824 and protein in urine of human nephrosis (17). Although it is not stated how dye was measured in nephrotic urine, the T-1824 clearance was 0.37 ml. per minute per square meter of body surface, the albumin clearance was 0.39 while total protein clearance was 0.09. The globulin clearance of 0.03 was very small indeed. Luetscher (17) preferred in 1944 to compare dye with total protein clearance, but since the clearances for T-1824 and serum albumin were nearly equal and considerably greater than that of globulin it is probable that the dye was excreted in the form of dye albumin. In nephrosis, then, the kidney behaves as though injected dye combined selectively with serum albumin leaving vanishingly small amounts of free dye in the blood. On the basis of body surface area a human nephrotic can have a dye clearance 100 times greater than that of a normal dog. This again suggests that dye clearance when analyzed by a sensitive method is a measure of normally occurring albumin clearance.

METABOLISM OF THYROXINE IN THE GOAT[1]

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DURING the past few years great progress has been made concerning the metabolism of the steroid hormones, especially the estrogens, androgens and progesterone. It has been shown that these hormones are metabolized by a variety of mechanisms in preparation for their elimination. The changes effected in the hormones—including the conversion to less active or inactive forms, and conjugation with glucuronidates or sulfates—are generally believed to occur in the liver.

The problem of the metabolism of thyroxine, on the other hand, has not received the study that its importance merits, due primarily to the fact that suitable methods for such investigations had not been developed until recently. However, many valuable inferences may be drawn from the older literature on iodine metabolism.

It may be said that, in general, the liver is primarily concerned with handling the more complex (organic) iodine compounds in contrast to the kidney, which is concerned mostly with the simpler (inorganic) iodine compounds. Consequently, the liver apparently plays an important rôle in the metabolism of the thyroid hormone as well as of the sex hormones. As early as 1919, Kendall (1) injected massive doses of thyroxine intravenously in the dog and found that 43 per cent of the iodine so administered was excreted in the bile in 50 hours. It is not known whether this iodine was in the form of thyroxine or of some decomposition product. A year later, however, Blum and Grützner (2) showed that the liver has the power to inactivate the thyroid hormone. Some of the earlier workers felt that the hormone was totally inactivated by the liver (3-5). However, their observations were based on rather insensitive biological tests. Zawadowsky and

Asimoff (6), on the other hand, suggested that the thyroid hormone is excreted by the liver unchanged. Their assay method (axolotl metamorphosis) was somewhat more sensitive, but not enough so as to make their observations conclusive.

Probably the truth of the matter lies somewhere between these two extremes. The work of Asimoff and Estrin (7) and Krayer (8) support the idea that thyroxine is only partially destroyed by the liver. Also, several investigators have shown, by chemical fractionation, that about one half to two thirds of administered thyroid hormone may be recovered in the bile (9-12). Kellaway *et al.* (13) are of the opinion that the thyroxine inactivating mechanism of the liver depends on the dosage of the hormone administered. Only when there is an excess of hormone, they say, is the destructive mechanism brought into play; and they stress the opinion that the mechanism is one of destruction rather than one of simple excretion. Presumably, on this basis, the amount of destruction would also depend on the dosage of the hormone administered.

Apparently, therefore, when thyroid hormone is administered to an animal, it may be partially broken down in the liver and partially excreted intact into the bile. As far as is known, no other organ or organ system contributes greatly to either the metabolism or excretion of thyroxine.

The recent work of Gross and Leblond (14), who used thyroxine labelled with radioactive iodine, bears out these postulations. In addition, these investigators found that much of the injected dose of thyroxine can be found *per se* in the feces, an observation which had been suggested previously (8, 15) but had not been emphasized in the literature.

Some results obtained at this laboratory also indicate that thyroxine is excreted intact in the feces by the goat. Since so little has been said on this subject in the literature, it seemed worthwhile to report our results at this time.

It was thought that the presence of thyroxine (or of some thyroidally active compound, at least) in the urine and/or feces could be detected by incorporating these excreta in chick rations simultaneously with thiouracil. The thiouracil, of course, would cause thyroid hypertrophy in the chick, due to the prevention of thyroxine synthesis and the consequent increase in thyrotrophic hormone secretion. It follows, therefore, that a material reduction of such hypertrophy would indicate the presence of a thyroidally active substance. An experiment was set up on this general hypothesis.

PROCEDURE

The urine and feces of 2 female, lactating goats were collected for five days. The urine was added to 10 kilos of poultry ration and the whole mixture dried in an oven at 45°C. The feces were dried at the same temperature, then ground and mixed in the feed at a level of 20 per cent by weight.

The goats were then injected subcutaneously with 10 mg. of D,L-thyroxine daily for a period of 10 days. This thyroxine was dissolved in N/10 sodium hydroxide and, therefore, was administered as the disodium salt.

The urine and feces were collected during the first five days of injections and were dried and mixed in the feed as described above. The same procedure was followed during the second five days of injections. Thiouracil[2] was added to each of these feeds at a level of 0.1 per cent by weight.

Eight groups of day-old White Rock chicks were used in this assay, each group consisting of about 20 chicks. One group received normal feed and served as a control. A second group received normal feed containing 0.1 per cent thiouracil. The remaining groups received the feeds containing the various samples of urine and feces described above (table 1). The assay was of three weeks' duration, starting on June 23 and ending on July 14. The chicks were kept in a basement room illuminated by incandescent bulbs and diffuse sunlight. The average daily temperature varied from 80-85°F.

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[2] We are indebted to Lederle Laboratories, Pearl River, N. Y., for the thiouracil used in this experiment.

At the end of the experimental period the chicks were killed by ether asphyxiation, their body weights ascertained and the thyroid glands removed and weighed immediately.   The sex of each chick also was determined at this time.

RESULTS

The results of this experiment seem relatively clear cut.   The addition of thiouracil to the normal chick ration, of course, caused marked thyroid enlargement (table

TABLE I.   THYROIDAL ACTIVITY IN THE FECES OF GOATS INJECTED WITH THYROXINE

GROUP	FEED	MALES				FEMALES			
		No.	Body wt.	Thyroid wt.	Thyroid wt/100 gm. body wt.	No.	Body wt.	Thyroid wt.	Thyroid wt/100 gm. body wt.
			gm.	mg.	mg.		gm.	mg.	mg.
I	Control	9	148.8	4.9	3.4 ± 0.9	12	159.3	7.9	5.0 ± 1.4
II	Control + 0.1% thiouracil	11	138.6	42.5	30.6 ± 10.8	7	132.1	48.7	36.9 ± 17.1
III	Control + thiouracil + 14.8 l. normal goat urine/10 kg. feed	9	121.4	25.0	28.9 ± 12.1	9	123.8	46.1	37.6 ± 10.9
IV	Control + thiouracil + 8.4 l. goat urine[1]/10 kg. feed	8	138.0	27.5	19.5 ± 8.1	12	143.2	52.0	35.8 ± 16.3
V	Control + thiouracil + 10.3 l. goat urine[2]/10 kg. feed	11	161.7	48.5	31.5 ± 11.9	8	146.4	57.0	39.3 ± 18.3
VI	20% normal goat feces + thiouracil	12	146.8	32.2	21.9 ± 13.2	7	143.7	49.9	35.0 ± 17.0
VII	20% goat feces[1] + thiouracil	7	153.7	37.4	23.1 ± 10.4	10	146.6	55.0	36.4 ± 20.9
VIII	20% goat feces[2] + thiouracil	10	145.9	8.2	5.8 ± 4.9	8	165.8	7.4	4.1 ± 2.1

[1] Collected during first 5 days of injections.   [2] Collected during second 5 days of injections.

1).   This hypertrophy was completely counteracted by the inclusion of goat feces collected during the second five days of the injection period.   By feeding this sample of feces to thiouracil-treated chicks, the thyroid glands of the chicks were maintained at normal weight.

On the other hand, the feeding of normal goat feces and feces collected during the first five days of injections to thiouracil-treated chicks seemed to cause only a slight reduction in thyroid size.   Moreover, this decrease is only apparent.   Statistical analysis showed that the thyroids of chicks thus treated were not significantly smaller than those of chicks receiving thiouracil alone.   The small decrease observed might well be accounted for by the fact that the nutritive value of the feed was probably lowered by the addition of feces.   Also, the chicks ate slightly less of the feed containing feces than did the chicks given normal feed.

Likewise, the inclusion of urine, from either normal or injected goats, did not cause a reduction of thyroid hypertrophy except in one group.   *Group IV*, male chicks, receiving thiouracil and urine (collected during the first five days of injections)

in their feed, had thyroid glands which, under statistical analysis, proved to be significantly smaller than the thyroids of male chicks receiving thiouracil alone. The female chicks receiving the same treatment were not likewise affected. Whether these results can be repeated remains to be seen.

DISCUSSION

Normally, the amount of total iodine excreted in goat feces is small—3 μg. in 24 hours (16). Assuming all of this iodine to be in the form of thyroxine, the feeding of normal goat feces at a level of 20 per cent by weight would be the equivalent of feeding 1.5–2.0×10^{-6} per cent thyroxine. Since it takes about fifty times that amount of crystalline D,L-thyroxine in the feed to maintain the thyroid glands of thiouracil-treated chicks at a normal weight (17), it is not surprising that we find no thyroidal activity in normal goat feces.

On the other hand, after thyroxine has been administered, even subcutaneously, there is an appreciable amount of the hormone passed into the feces. As shown above, enough hormone is present in the feces, collected during the second five days of injections, to maintain the thyroids of thiouracil-treated chicks at normal weight when the feces comprise 20 per cent of the feed. The same result can be effected by feeding the disodium salt of D,L-thyroxine[3] at a level of 3.5×10^{-5} per cent by weight (17). Therefore, it can be calculated that these feces contained approximately the equivalent of 0.35 μg. of disodium D,L-thyroxine per gram of dried feces. On this basis, the daily excretion would be roughtly 0.075 to 0.1 mg. of thyroxine per goat. This amount accounts for only one per cent or less of the daily injected dose. Moreover, subsequent experiments, although not conclusive, seem to indicate that the hormone is excreted at this rate for only a short time after the injections are stopped and that hormone excretion probably ceases altogether after a few days.

Obviously, much of the injected thyroxine remains to be accounted for. Presumably it is broken down in the body, probably in the liver for the most part. The mechanisms which the liver employs to accomplish this inactivation of thyroxine can only be speculated upon at this time.

Perhaps, since thyroxine is an amino acid, its inactivation follows the usual path of amino acid metabolism; i.e., deamination (probably oxidative), which might or might not be followed by a secondary reduction. The end product would then be an α-keto acid or, if the secondary reduction did take place, an α-hydroxy acid. Some credence is lent to this possibility by the observation of Foster and Gutman (19) that after the administration of massive doses of diiodotyrosine, a compound identified as 3,5-diiodo-4-hydroxyphenyl, lactic acid was found in the urine.

Other possible methods of thyroxine inactivation include: the release of iodine from the thyroxine molecule (12, 14) to form the inactive thyronine; the conversion of the physiologically active levorotatory isomer to the inactive dextro-isomer (20); and the breaking of one (or maybe even both) of the thyroxine rings (21).

At any rate, the amount of thyroidally active material found in the feces in these experiments is much smaller than the amounts reported by other workers (8, 14). Krayer (8), of course, based his findings on the increment in fecal iodine after the injection of thyroxine; so we have no way of knowing, from his work, how much biologically active material was actually excreted. Gross and Leblond (14),

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[3] Prepared by the method of Harington (18).

on the other hand, used thyroxine labelled with I^{131} and found that 80 per cent of the injected dose of radioactive thyroxine appeared in the feces in 24 hours. About one half of this amount was butanol soluble; i.e., was present as thyroxine. This recovered thyroxine, however, was found to be somewhat less active biologically than thyroxine prepared by the investigators or commercial preparations. They attributed this fact to either the presence of an inactive iodine compound dissolved in the butanol or to a toxic effect of the feces extract, which might cause a diminished metabolic response to thyroxine. It would be interesting to determine how much of the thyroxine is excreted in the d-form (inactive).

Concerning the mechanism of fecal excretion of thyroxine, there seem to be two major possibilities: *a*) the liver probably excretes some unchanged thyroxine into the bile, and hence into the digestive tract (8–12, 14); and *b*) the intestine may actively excrete some thyroxine (15). The former route is doubtless the more important (14).

It must be remembered, however, that the gastrointestinal tract also absorbs thyroxine. Probably the actual, overall picture is a complicated combination of excretion, reabsorption, and metabolism, the extent of each of these processes depending on various unknown factors.

SUMMARY

A study has been conducted on goats to determine whether thyroxine injected subcutaneously is excreted in the feces and/or urine in a biologically active form. It was found that unmistakable activity appeared in the feces of goats during the fifth through the tenth day of a 10-day injection period. Estimations of active material in the feces are presented in terms of the amount of disodium thyroxine required to evoke an equivalent response. No activity could be detected in normal goat feces or urine, in feces collected during the first five days of injections, or in urine collected at any time during the injection period.

Various possible mechanisms of thyroxine inactivation are discussed.

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INTERRELATION BETWEEN THE LENGTH OF SYSTOLE, STROKE VOLUME AND LEFT VENTRICULAR WORK IN THE DOG[1]

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I T IS well established that for resting human subjects, the Q-T interval of the electrocardiogram varies directly with the length of the cycle (1–4). Hence there seems to be a basic relation between the duration of the excitation process, and the frequency of pacemaker discharge. During periods of rapid change in cycle length, however, the relationship is less exact (4). When the duration of the actual ejection period is plotted against cycle length, for normal dog hearts in a wide variety of conditions, the correlation between the lengths of systole and cycle is very rough indeed (fig. 1). Factors other than cycle length must play a rôle in determining the length of the ejection period. Thus Wiggers, and Wiggers and Katz (5–7), have shown that an increase in filling pressure (right auricular pressure at the end of diastole) tends to increase the relative duration of systole and that an increased arterial pressure and sympathetic stimulation tend to decrease it. These observations were made on the exposed heart whose systolic duration was very much longer than that of the heart beating in the closed thorax. It was thought advisable, therefore, to inquire further into the factors affecting length of systole in the intact dog.

In a previous publication (8), we have shown that what can be learned from the heart lung preparation applies also to the intact animal, namely that an increase in arterial pressure serves to decrease the stroke volume and often the external cardiac work. Conversely, a fall in arterial pressure produces an increase in stroke volume. It was also confirmed for the intact animal that, within the limits set by the pericardium, a rise in venous pressure increases both stroke volume and external work. In the absence of sympathomimetic myocardial stimulation, which shortens the duration of systole, the data of Wiggers (5–7) would suggest, therefore, a possible parallelism between the length of systole, the stroke volume and external ventricular work.

As a first step in the delineation of the factors causing a change in length of systole in the intact animal, statistical analyses were made of values derived from some 800 dog aortic pressure pulse contours, taken from a number of animals, and chosen to represent a rather wide variety of experimental conditions. The lengths of systole were measured as the time from abrupt pressure rise to the incisural notch. These reference points were taken for convenience only. The length of systole so

recorded is, of course, not an index to the duration of muscle contraction, for the iso-metric contraction period is not included.   Neither does it coincide with the ejection period, for outflow from the ventricle may cease before the incisura.   While this time difference is not necessarily constant, it is small so that, roughly, the length of systole as measured will reflect changes in the duration of ejection.

  The length of systole values (Ts) were plotted against the respective cycle lengths (fig. 1 A). The points are widely scattered about a curved line that was plotted from average Ts values within cycle length groups.   This curve is not fitted, over its whole length, by exponential equations similar to those used to relate the length of electrical systole to cycle length (1, 4).   The correlation coefficient for the data of figure 1 A is only 0.62.   The curved relation renders most questionable the significance of this figure.   The correlation coefficient with the square root of cycle length, or with the reciprocal of cycle length, i.e., pulse rate, is higher (r = 0.75; 0.77).
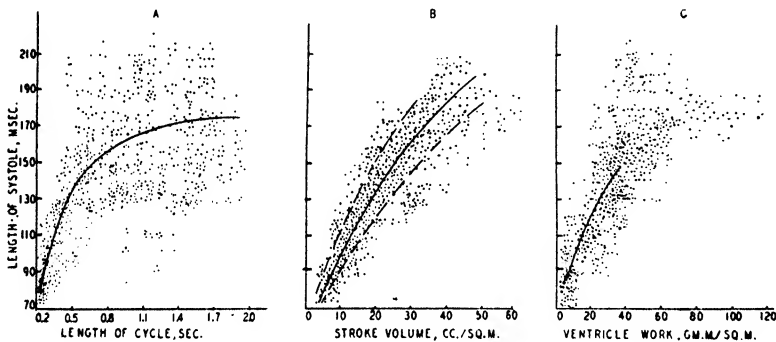
Fig. *1A*. RELATION BETWEEN LENGTHS OF SYSTOLE AND CYCLE LENGTHS of dog pulses.   *1B*. Relation between lengths of systole and stroke volumes for the same series of pressure pulses.   Dotted lines represent 25 per cent deviation from the average curve.   *1C*. Relation between lengths of systole and work of the left ventricle.

The scatter of the points is greater than that recorded for electrical systole. This is partly because heart rate changes are greater and physiological conditions more variable in the present series.   Moreover, electrical criteria measure only the duration of the process of excitation of the myocardium.   The length of mechanical systole, while it has a similar trend to that of Q-T, is modified by conditions imposed upon the heart as it attempts to eject blood against a variable level of aortic pressure (5-7).

  In other words, under certain conditions there is an even greater shortening of mechanical systole than a cardio-acceleration will account for.   Also, when the heart is slowed, the length of systole may remain the same or lengthen with the cycle length.   As a measure of this dispersion, we have taken the average curve of figure 1 A as the comparison standard (table 1) and divided the actual length of a particular systole by the value expected from the length of the cycle, expressing the quotient as a percentage (Ts/Ts$_e$ × 100).   This figure will express the effect of mechanical variables upon Ts, discounting in advance the effect of heart rate changes.

  It should be emphasized that the figure for Ts/Ts$_e$ is of significance only as it changes.   It represents the relation of an actual Ts to an average at that particular

heart rate. This average is derived from pulses from different physiological states and is weighted heavily by pulses from partly deteriorated animals. In 43 newly anesthetized animals, Ts/Ts$_e$ averaged 113 with a standard deviation of 10. As the animal deteriorates during an experiment, this value falls progressively to 100, or below. Likewise, an animal in shock shows low values (11).

The observation of Wiggers that increased aortic pressure decreased the duration of systole justifies inquiry as to whether blood pressure is itself one of the factors which produced trends away from the average in figure 1 A. The correlation between Ts and the height of systolic and diastolic pressure levels was found to be 0.28 and 0.36 respectively. It would seem, therefore, that the effect of pressure level on systole length was not a primary relation. On the other hand, the correlation between Ts and stroke index of these dogs, either as calculated from the pulse contour (9) or as obtained by the dye injection technique (10), is quite high (r = 0.82). The relation of the individual measurements is shown in figure 1 B. The scatter is not large, and the stroke index can be predicted from Ts with an average error of 25 per cent.

TABLE 1. AVERAGE RELATION BETWEEN LENGTH OF CYCLE AND LENGTH OF SYSTOLE

LENGTH OF CYCLE	LENGTH OF SYSTOLE (Ts$_e$)	LENGTH OF CYCLE	LENGTH OF SYSTOLE (Ts$_e$)	LENGTH OF CYCLE	LENGTH OF SYSTOLE (Ts$_e$)	LENGTH OF CYCLE	LENGTH OF SYSTOLE (Ts$_e$)
msec.	*msec.*	*msec.*	*msec.*	*msec.*	*msec.*	*msec.*	*msec.*
250	84	500	134	750	155	1200	168
300	99	550	141	800	157	1400	172
350	110	600	145	900	161	1600	174
400	119	650	149	1000	164	1800	175
450	127	700	152				

A similar correlation obtains between Ts and pulse pressure (0.79) and between Ts and left ventricular external work (0.86) (fig. 1 C). The high correlation with pulse pressure may be regarded as a corrolary of the high correlation with stroke volume. The high correlation with external work may be either direct or indirect through the relation with stroke volume. A case could be presented for either view point. For example, if the above series be subdivided into two groups, those with high and those with low diastolic pressure values, the correlation between Ts and stroke index is essentially the same for both groups. That, between Ts and work, however, is definitely better (r = 0.92) for the high-pressure pulses and definitely inferior (r = 0.63) for the low-pressure pulses.

For an analysis of this difference with pressure levels, we have followed the immediate changes in stroke volume, work and Ts/Ts$_e$ when cardiodynamics were abruptly altered by various experimental procedures.

From table 2 A-C it is seen that when the abdominal aorta, just below the diaphragm, was occluded the stroke volume, work and Ts were at first reduced. As the occlusion persisted all three might return toward the initial level. With marked cardiac slowing, the work and Ts/Ts$_e$ might exceed the initial value (2 C). In other cases, all quantities might remain low throughout the occlusion (2 B).

From the average stroke volume and Ts relation (fig. 1 B), one can predict the stroke volume which should correspond to each Ts value.   From such values (table 2), it is apparent that, when the heart is faced with a high aortic resistance, the stroke volume is curtailed to a much greater degree than is Ts.   Not only did Ts/Ts_e decline less than stroke volume or work but also the change was of briefer duration, so that Ts/Ts_e might return to the initial value while the other measures were still reduced (table 2 A).

A number of constrictor drugs which, in our hands, have had a minimal effect upon the heart gave similar changes in Ts, work and output.   As the pressure first rose after the injection of angiotonin[2] (table 2 D) or Privine (table 2 E), the stroke volume and work were reduced and Ts/Ts_e was shortened slightly.   Later the work returned toward normal and Ts/Ts_e lengthened, even though there had been no increase in stroke volume.   After Neosynephrine (table 2 F) neither work nor Ts/Ts_e decreased, even though the pressure was increased and the stroke volume decreased.

In all these experiments in which the heart was suddenly faced with an increased aortic resistance to ejection, Ts, stroke volume and work were immediately curtailed. After this initial transient change, Ts returned to the expected value and then varied with cycle length but not with stroke volume or work.

Another group of sympathomimetic drugs which bring about their pressor effects by increasing the cardiac output allow a definite increase in Ts/Ts_e.   Thus Priscol (table 2 G) and ephedrine (table 2 H) produced a decided increase in work per beat and in Ts/Ts_e.   In some cases there was a moderate increase in stroke volume as well. In all these cases, the changes in Ts/Ts_e paralleled those of work more than those of stroke volume.

When the aortic pressure was suddenly reduced, the work might or might not be increased as the stroke volume was increased.   For example, when an aortic occlusion was released (table 3 A), or when a shunt between the abdominal aorta and vena cava was opened (table 3 B), both stroke volume and Ts/Ts_e were abruptly increased. Work changes were rather variable, depending upon the pressure levels.   In the first case, for example, the work was also momentarily increased; in the second, it was not. The limitation of cardiac ejection with a low aortic pressure depends primarily on the adequacy of venous filling rather than aortic resistance and the ventricle can easily eject its whole possible stroke volume.   The presence of a reactive hyperemia in the peripheral beds, for example, allows less of an increase in Ts than does the opening of an A-V shunt or the injection of acetylcholine.   The fact that the external work may not numerically reflect the increased ejection, and hence be no criterion to Ts/Ts_e, need not be surprising.

With extremely long cycle lengths, as during the stimulation of the peripheral end of the vagus (3 C), Ts might remain relatively independent of cycle length, and Ts/Ts_e thereby be reduced.   Ts itself was lengthened as the stroke volume increased and in about the ratio expected from figure 1 B.   Both values reached the maximum available for this animal under the existing conditions and, beyond a certain level, a

TABLE 2

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	T_s/T_{s_0}	EXPECTED[1] STROKE INDEX
		A—Aortic occlusion, after atropinization					
sec.	mm. Hg	cc.	gm. M.	msec.	msec.	%	cc.
0	126/ 93	18	30	120	400	101	15
3	186/154	5	14	110	400	93	13
5	196/162	6	15	115	400	96	14
10	195/160	6	15	117	400	97	14
		B—Aortic occlusion					
0	102/ 77	21	26	140	390	120	22
3	200/156	4	10	125	390	107	17
5	210/163	8	20	140	420	115	22
10	210/158	9	20	150	510	112	24
		C—Aortic occlusion					
0	140/118	18	33	140	600	97	22
2	170/140	15	27	130	580	90	18
5	264/160	16	50	180	800	115	37
10	240/160	12	38	160	800	103	28
		D—Angiotonin, 10 units					
0	110/ 88	14	19	125	350	113	25
5	140/126	11	17	115	350	104	24
10	148/134	10	20	120	340	111	32
15	152/134	11	20	127	340	117	27
		E—Privine, .02 mg/kg. B.W.					
0	162/132	13	22	90	270	100	8
10	204/166	7	15	86	280	90	7
20	190/150	8	16	95	270	105	9
		F—Neosynephrine, .02 mg/kg. B.W.					
0	140/116	13	17	130	440	104	18
10	176/148	10	17	140	480	109	21
		G—Priscol, 10 mg/kg. B.W.					
0	164/135	13	28	150	580	104	24
10	176/140	15	35	160	600	110	28
30	184/145	17	38	165	620	113	30
50	193/147	18	42	170	620	116	32
		H—Ephedrine, 5 mg/kg. B.W.					
0	130/104	16	25	90	280	95	9
5	140/116	15	27	90	270	100	9
10	156/120	16	32	100	240	120	10
15	158/122	17	32	110	280	114	13

[1] Stroke index expected from length of systole (fig. 1 B).

further increase in diastolic time did not produce an appreciable increase in either factor.

When low arterial pressure was produced by acetylcholine injection (3 D, E), Ts tended to increase as the stroke volume increased, while the work changes were

TABLE 3

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	Ts/Ts:	EXPECTED STROKE INDEX
			A—Release of aortic occlusion				
sec.	*mm. Hg*	*cc.*	*gm. M.*	*msec.*	*msec.*	*%*	*cc.*
0	226/176	8	20	115	430	93	15
5	122/106	33	43	130	420	107	18
10	110/ 92	27	27	124	410	103	17
15	100/ 84	25	25	120	400	101	16
			B—Abdominal aorta—vena cava shunt				
0	128/ 92	24	22	170	550	120	33
1	62/ 40	40	16	190	550	135	42
2	82/ 28	42	14	198	560	141	46
3	86/ 36	48	18	200	570	141	47
			C—Stimulation of peripheral end of vagus nerve				
0	70/ 48	11	9	82	250	98	6
5	75/ 20	29	30	138	1250	82	21
10	92/ 30	36	33	140	1750	80	21
15	82/ 38	29	25	130	1250	77	18
			D—Acetylcholine, .004 mg/kg. B.W.				
0	120/ 95	21	30	125	340	110	17
2	100/ 70	23	26	120	320	117	16
5	90/ 58	27	29	130	290	136	18
			E—Acetylcholine, .02 mg/kg. B.W.				
0	105/ 80	20	25	120	360	107	16
2	64/ 20	30	17	160	900	100	28
5	76/ 28	33	23	160	690	106	28
8	82/ 58	26	25	140	290	142	21

variable. Just as with vagus stimulation, with very long cycle lengths, Ts/Ts_e might be reduced.

As shown by Wiggers (6), sympathetic stimulation of the heart gives rise to an abridgement of systole. The stimulation of the sympathetic nerves to the heart (table 4 A) gave a cardio-acceleration and increased stroke volume and ventricular work, but a decreased Ts/Ts_e. This effect is directly opposed to the Ts/Ts_e response to be expected when the work per beat is increased. Similarly, injected epinephrine gave a reduced Ts/Ts_e (table 4 B). As we have shown (8), the usual response to epinephrine in the dog is to reduce the stroke volume and external work, since the vasoconstriction outweighs the cardiac stimulation.

TABLE 4

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	Ts/Ts_0	EXPECTED STROKE INDEX
A—Stimulation of cardiac nerves							
sec.	mm. Hg	cc.	gm. M.	msec.	msec.	%	cc.
0	170/134	15	31	148	460	116	24
5	190/146	16	38	136	430	109	20
10	200/154	18	43	132	425	107	19
20	198/148	15	35	135	435	108	20
B—Epinephrine, .005 mg/kg. B.W.							
0	143/115	20	37	120	400	101	16
5	156/130	11	20	95	320	92	9
10	168/137	10	22	108	600	75	12
15	160/118	17	35	150	800	96	24
25	132/107	21	38	130	500	97	18
35	130/101	24	40	130	400	109	18
C—Epinephrine, .002 mg/kg. B.W.							
0	165/108	27	50	170	900	106	33
10	168/108	27	50	120	760	78	15
20	176/108	31	58	130	660	88	18
D—Epinephrine, .015 mg/kg. B.W.							
0	133/105	22	36	140	370	123	21
5	235/180	13	37	120	350	109	15
10	300/210	14	51	140	350	127	21
E—Epinephrine after priscol, .005 mg/kg. B.W.							
0	180/138	17	39	148	520	109	24
10	209/145	17	44	110	490	83	13
20	170/115	25	46	100	380	87	11
30	148/104	27	49	90	400	84	11
F—N-isopropyl arterenol, .0025 mg/kg. B.W.							
0	150/100	26	44	140	420	116	21
5	156/ 90	33	55	110	350	100	13
10	143/ 70	47	65	100	280	107	11
15	130/ 60	29	37	90	260	103	8
G—N-isopropyl arterenol, .0002 mg/kg. B.W.							
0	165/122	16	33	140	520	103	21
5	167/125	16	33	100	360	89	11
10	158/118	15	30	100	350	91	11
15	166/125	16	33	110	420	91	13
20	165/125	16	33	130	470	99	15

Cases where the stroke volume reduction is minimal, or absent, (4 C) still show an abridgement of Ts/Ts$_e$. That this shortening is due to a direct action of the drug, and not to the mechanical effect of the rise in pressure, was shown by several experiments. In some cases, the effect upon the blood pressure (and hence upon the stroke

Fig. 2. RELATIONS between pressure pulses, derived ejection curves and work curves for dog pulses in various physiological conditions. *A*—Before (*solid*) and after (*broken*), the injection of epinephrine. *B*—Before (*solid*) and after (*broken*) the opening of an aorta-vena cava shunt, at the height of the pressure rise following the injection of epinephrine. *C*—Before (*solid*) and after (*broken*) the injection of isopropyl arterenol. *D*—Before (*solid*) the occlusion of the abdominal aorta, during (*broken*) that occlusion, and immediately after (*dotted*) the release of the occlusion. *E*—Before (*solid*) and after (*broken*) the injection of Priscol. *F*—Before (*solid*) and after (*broken*) the injection of angiotonin.
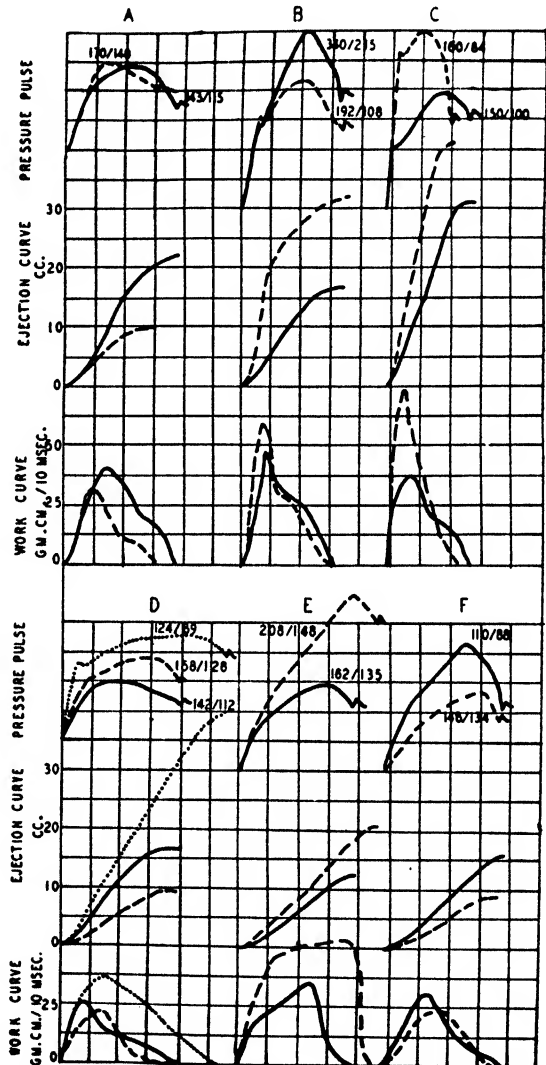
volume and work output) outlasted that upon systolic length, which might return to its pre-injection value even though the stroke volume was still curtailed by the high pressure (4 D). When, at the height of the pressure rise, the peripheral resistance was suddenly lowered by the opening of a previously established shunt between the aorta and vana cava, the length of systole did not increase, although the stroke vol-

ume and external work were increased to a great degree. Small doses of epinephrine might cause a reduction in Ts/Ts_e even when there was no effect upon the blood pressure or blood flow (table 4 C). When epinephrine was given after Priscol, a drug which blocks the vasoconstrictor action, the length of systole was still reduced (table 4 E) even during the period in which the stroke volume was increased and the blood pressure was falling.

N-isopropyl arterenol, which possesses the cardiac actions of epinephrine, but is a peripheral vasodilator (8, 12) will, in minimal doses, give a reduction in the length of systole, a cardio-acceleration, and no change in blood pressure or stroke volume (table 4 G). In larger doses, it curtailed Ts/Ts_e in spite of an increase in stroke volume and ventricle work (table 4 F).

Hence the reduction in Ts seen after cardiac nerve stimulation, or use of epinephrine-like compounds, is due to a direct action on the time course of contraction and is not to be attributed to the influence of mechanical factors upon the performance of the ventricle. This is shown graphically by the cardiac ejection curves which can be constructed from the aortic pressure pulses (13). For example, in figure 2 A are given representative pulse contours taken before and during the first part of the response to epinephrine, with their derived ejection curves, and also the curves depicting the work done during the course of systole (14). The changes are quite similar to those obtained by Wiggers with a cardiometer when epinephrine was given (6). After the drug, the time course of ejection was altered, so that the maximum output came earlier in systole, and both ejection and external work fell off rapidly during the last two thirds of the ejection period. The myocardial stimulation is hence revealed in a more forceful initial contraction, but a contraction which can be but poorly sustained.

That the changes pictured cannot be attributed simply to the reduced stroke volume and external work is evident from figure 2 B and C. In figure 2 B a previously established A-V shunt was opened at the height of the pressure rise after the injection of epinephrine. As the pressure was suddenly lowered, stroke volume and work were increased, but the contour of the work curve showed but minor variation. In figure 2 C, N-isopropyl arterenol was used to produce the cardiac stimulation. Both stroke volume and cardiac work were enchanced. Despite this, the maximal ejection rate and work production were reached earlier in systole after its use, and, like the response to epinephrine, both work and ejection were but poorly sustained toward the end of systole.

On the isolated heart, the actions of these two drugs are identical. In the intact dog, N-isopropyl arterenol, being a peripheral dilator instead of constrictor, always increases the stroke volume. Epinephrine, because of its intense peripheral constriction, usually leads to a decreased stroke volume in the dog.

Work contour changes which follow a rise in aortic pressure do not show the above changes. After the application of an aortic occlusion (fig. 2 D), or the injection of angiotonin (fig. 2 F), there was a broadening of the work curve, with the peak falling later in systole. Changes which followed the release of an aortic occlusion (fig. 2 D), or the injection of Priscol (fig. 2 E), were quite similar. In these cases, the cardiac work was increased rather than decreased, Ts/Ts_e was lengthened and

the ventricle actually worked strongly for almost the whole of the ejection period. The pressure pulse contours reflect this change, showing a pressure peak late in systole. After epinephrine, the pressure peak is reached in mid- or early systole.

SUMMARY

1. Mechanical factors upset a natural relation between length of systole and cycle length. Hence length of systole shows good correlation with stroke volume and with left ventricle work in the dog. 2. Systole is longer than might be expected from cycle length when the arterial pressure is low and venous return to the heart is adequate. This increase parallels the increase in stroke volume. Systole is also prolonged when the heart is stimulated by ephedrine or Priscol to do more work. 3. Systole is shorter than might be expected from cycle length, very transiently, when there is a sudden increase in aortic resistance to ejection. A more prolonged curtailment of systole follows cardiac nerve stimulation, or the injection of epinephrine or related compounds. This change reflects a basic change in the ejection curve.

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CHOLESTEROL AND CAPILLARY PERMEABILITY[1]

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MUCH past research has ascribed to cholesterol the function of regulating cellular permeability (1–4), but these claims were largely inferential, or based on evidence from work with single cells or isolated tissues. It was our purpose to see if cholesterol has any effect on capillary permeability, as tested in a total mammalian animal.

PROCEDURE

Changes in capillary permeability were tested by the transfer of intravenously injected dye, T-1824, from the blood to the lymph, and also by the disappearance rate of the dye from the blood stream. Twenty-four dogs were used, comprising 10 untreated controls, 4 controls treated with water and 10 experimental animals treated with cholesterol.

All finally underwent the same procedure. The dog, fasted for 20 hours, was intravenously anesthetized with nembutal, 30 mg./kg. and the thoracic duct exposed and cannulated. The lymph was collected continuously in test tubes and the mean time of collection was noted for each test tube. After control specimens of blood and lymph were obtained, a measured amount of dye was injected into the exposed femoral vein. Lymph specimens were timed at 5, 15, 30, 45, and 60 minutes following dye injection. Blood samples from the contralateral femoral vein were taken at 15, 40, and 60 minutes after dye injection.

METHODS

Treatment of blood and lymph specimens. Blood samples were centrifuged immediately after withdrawal, the relative amounts of cells and clotted plasma measured for hematocrit determination, and serum expressed by pressure. Lymph volumes were noted and fluid lymph expressed from the clot. All serum and lymph specimens were then treated with alcoholic phosphotungstic acid (5) to remove proteins and lipids. The dye content of these samples was determined by a photelometer. Total serum cholesterol was determined by the Leiboff modification of the Myers-Wardell method (6).

Administration of cholesterol. Cholesterol suspensions were made following the method of Cole, Clarke and Womack (7). In some cases sodium laurylsulphonate was used as a wetting agent in order to produce a finer suspension. This proved ir-

ritating to the tissues and was discontinued. The experimental dogs received 40 to 60 cc. of suspension of a strength approximately 2.5 gm. per cent, intraperitoneally, each day for one week preceding the operation. Sterile precautions were observed.

Administration of water. Four dogs received the same treatment as the experimental dogs except that they received a mock 'suspension' of water in place of the cholesterol suspension.

RESULTS

Cholesterol findings. The control range of serum cholesterol in the untreated group was 43 to 196 mg. per cent, in the water-treated group 50 to 102 mg. per cent before the administration of water. For the experimental animals before treatment, this value was 45 to 148 mg. per cent. The entire group of 21 control determinations presented a range of 43 to 196 mg. per cent, with a mean of 95 mg. per cent, and

TABLE 1.   EFFECT OF CHOLESTEROL ON VARIOUS FUNCTIONS RELATED TO CAPILLARY PERMEABILITY

FUNCTION TESTED	CONTROL GROUP			EXPERIMENTAL GROUP		
	No. of cases	Mean	s.d.	No. of cases	Mean	Dev. from control mean
Dye disappearance rate (%)	14	6.6	4.5	10	6.2	−0.4
Blood vol. (cc/kg.)......................	13	90	9.1	10	85	−5.0
Hematocrit (%)........................	14	44	6.2	10	46	+2.0
Lymph flow (cc/kg.)....................	14	.035	.023	9	.031	−.004
Transfer of dye to lymph:						
a. Time of maximum dye conc. in lymph (min.)................................	14	43	14.8	10	33	−10
b. Maximum dye conc. in lymph/plasma dye conc. (15′)...........................	14	.414	.180	10	.433	+.019
c. Total dye in lymph (60′)/plasma dye conc. (15′)................................	14	.082	.072	10	.096	+.013

standard deviation of 35.6 mg. per cent. Glusker (8) found a standard deviation of 25 per cent for a series of normal dogs.

The 4 dogs treated with water evinced essentially no change in serum cholesterol. Before and after treatment the percentage change in cholesterol for each animal ranged from −2 to +9 per cent, the mean percentage change being +2 per cent, with a standard deviation of 4.6 per cent. Glusker gave 6 per cent as the standard deviation for 4 determinations on single dogs.

The 10 dogs treated with cholesterol evinced a rise in serum cholesterol in each case. The experimental range was 77 to 208 mg. per cent, with a mean of 156 mg. per cent, exhibiting a difference of +61 mg. per cent over the control mean of 95 mg. per cent, a difference almost twice as great as the standard deviation. The percentage change in cholesterol level for each case ranged from +8 to +276 per cent. The mean change was +76 per cent, to be compared with the mean change of +2 per cent for the water-treated animals. This difference was 16 times as great as the standard deviation, so that the rise in serum cholesterol after administration of cholesterol was unquestionably significant.

Experimental findings in regard to capillary permeability. In the case of all the functions investigated, the findings were negative. There was no statistically significant difference between the control group of untreated dogs plus water-treated dogs and the experimental group of cholesterol-treated dogs in regard to; *a*) disappearance rate of the dye from the blood stream, *b*) blood volume, *c*) hematocrit, *d*) lymph flow, *e*) transfer of dye to the lymph, either in regard to time of appearance, concentration achieved, or absolute quantities recovered in the lymph (table 1).

The correlation between individual cholesterol levels and dye disappearance rates was low (r −.21). The correlation between individual cholesterol levels and dye transfer to the lymph was absent (r +.077).

DISCUSSION

The method was found in the following paper (9) to be adequate for detecting marked changes in capillary permeability. In this case the experimentally induced rise in serum cholesterol, while not drastic, was of an order which might reasonably be expected to produce changes, if these were to be found. The consistently negative results found in this work, together with the failure of other investigators to find direct evidence of a dynamic effect of cholesterol on permeability, seems to lead inevitably to the negative conclusion.

SUMMARY

Intra-peritoneal injections of cholesterol suspension raised the plasma cholesterol level of dogs. An increased plasma cholesterol level had no effect on capillary permeability, as tested by the disappearance rate of dye from the blood stream and the transfer of dye from blood to lymph. In addition, no effect was found in the case of blood volume, hematocrit and lymph flow.

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VITAMIN D AND THE DISAPPEARANCE OF T-1824 FROM THE BLOOD[1]

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MANY substances are known to increase the permeability of capillary endothelium (1–5). Substances which can counteract this process, producing a condition of decreased permeability of capillaries to large water-soluble molecules, are less well known. Calcium and the adrenal cortical hormones (7–9) are of the latter category, and preliminary experiments in this department (10) indicated that vitamin D might also be included. Later work was confirmatory, both as to substance and method, and the final results are reported here.

METHODS

Adult male dogs weighing 10 to 20 kg. were used. The disappearance rate of the dye T-1824 was used as the indicator of capillary permeability. Dye disappearance rate was determined by a technique similar to routine determinations of plasma volume and was calculated as the percentage difference in dye concentraion between 15 and 60 minutes after dye injection.

In acute experiments, where lymph from fasting dogs was analyzed for dye content, the collected lymph was allowed to clot, compressed to express the dyed fluid, and the specimens then read on a photoelometer exactly as were the serum samples, since under these conditions the optical densities of serum and lymph were similar.

Vitamin D was administered orally in capsules of 50,000 International Units (IU). When histamine was employed, it was injected intravenously, using 1.5 mg/kg. of body weight.

Effect of Vitamin D on Capillary Permeability

Procedure and results. Trained unanesthetized dogs were tested in several con-trol determinations of dye disappearance rate, plasma volume and hematocrit. Vitamin D was then administered, and the determinations were continued for a vary-ing period thereafter. The preliminary work had shown that lower or less concentrated doses of the vitamin had no uniform effect. Doses used had been *a)* 11,000–16,000 IU/kg/day for one to four days, and *b)* 2500–11,000 IU/kg/day for 40 days.

Positive results were obtained when a total dose of approximately 100,000 IU/kg. was administered over one to seven days. This dose was administered to 18 dogs, and repeated one week later in the case of 12 of the animals. The experimental

changes described below were detected in the determinations which were made within a period of from five to eight days after discontinuing the dosage. Both before and after this period, the experimental values approached the control level.

TABLE 1. EFFECT OF MASSIVE DOSE OF VITAMIN D

CASE	CHANGE IN BODY WT.	CHANGE IN DYE DISAPPEARANCE RATE	CHANGE IN PLASMA VOL.	CHANGE IN HEMATOCRIT	CHANGE IN BLOOD VOL.
	%[1]	%[1]	%[1]	%[1]	%[1]
4650a	+5	−125	+7	+8	+13
4848a	+3	−100	−6	+7	−4
4656a	−2	−180	−4	+7	+2
7	−4	0	−5	+10	+5
3	−7	−114	+60	+6	+71
4848b	−7	+50	−7	+19	+2
2a	−8	−87	+22	+15	+45
8	−8	−200	+4	+11	+15
4653a	−9	−67	−5	+17	+8
4654a	−9	−100	−21	+13	−11
4658b	−9	+33	−17	+16	0
9a	−9	0	−	+16	−
4659b	−10	−40	+18	+12	+28
4650b	−11	−75	−20	+28	−3
4655a	−11	−69	−23	+14	−10
4734a	−11	−	−14	+15	+3
4	−13	−170	+39	+16	+43
6	−13	−743	+23	+12	+35
4656b	−13	−80	−16	+25	+4
4659a	−13	−50	−8	+7	−4
4658a	−14	0	−3	+10	+8
2b	−16	−127	+25	+33	+71
9b	−17	+115	−28	+24	−16
4652a	−18	−44	−4	+21	+5
4654b	−18	−29	−21	+22	−4
4655b	−18	−62	−24	+27	+7
4653b	−19	−67	+1	+33	+33
5	−20	+218	−39	+11	−34
4734b	−23	−57	−42	+21	−13
4652b	−24	−28	−15	+64	+14
MEAN.......	−11.5	−66	−4	+18	+11

Single dose of 100,000 IU/kg., followed by latent period of one week (approx.).

[1] Based on deviation of experimental datum from last control datum. Omitting two extreme values.

It was found that vitamin D administration was followed by a decrease in capillary permeability, as measured by the dye test. Table 1 gives the percentage change in the various functions found after the latent period, the experimental datum in each case being compared to the last control datum before dosing. Out of a total of 29 tests of dye disappearance rate, 22, or 76 per cent showed a decrease in the rate at

which dye left the blood stream. The mean percentage change in disappearance rate, omitting two extreme values, was −66 per cent.

There was a rise in the hematocrit value in each of the 30 cases tested (mean increase was +18%). This rise was not solely a passive reflection of plasma loss, as may be seen from the fact that the blood volume did not decrease, but rather the reverse (mean change in blood volume was +11%). Twenty cases showed a decrease in plasma volume, and 9 cases an increase (mean change was −4%). The
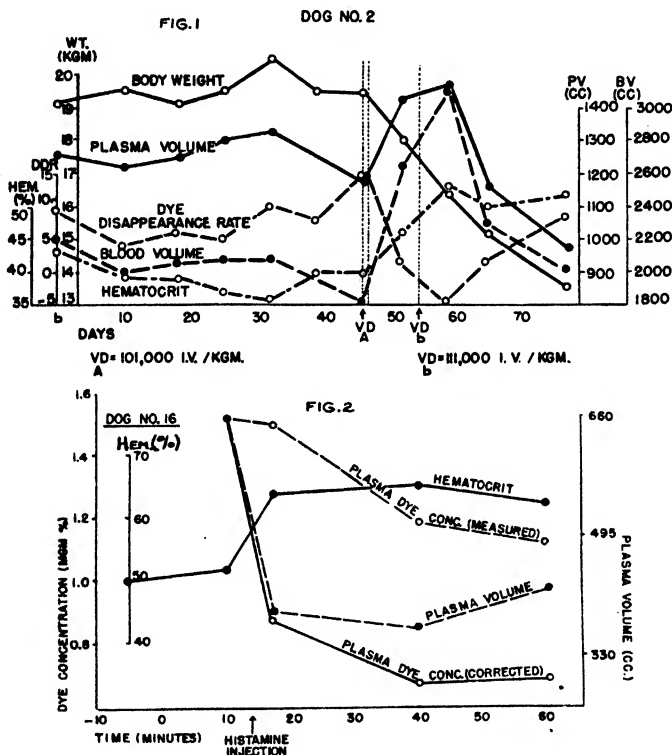
Fig. 1. Typical effect of concentrated dose of vitamin D (approximately 100,000 i.v./kg.).

Fig. 2. Histamine experiment on unanesthetized dog showing effect of plasma loss on plasma dye concentration. Correlations calculated according to text. Dye injected at o time.

body weight decreased in 28 out of the 30 cases (mean change was −11.5%). Figure 1 illustrates the findings on a typical animal.

In table 1 the data are arranged by degree of weight loss and it is apparent from inspection that the rise in hematocrit is related to the loss in weight (correlation coefficient, r, was found to be −.63). The change in dye disappearance rate showed no correlation with weight loss, however (r was −.29).

Discussion. The results indicated that a high dose of vitamin D produced decreased capillary permeability in dogs. This dose also caused anorexia and a decreased water intake on the part of most of the dogs, which was reflected in the loss of body weight over the experimental period. The consistent finding of the rise in

hematocrit associated with the loss in weight is in accord with the findings of DeBoer (11) on fasting, aquaprivic dogs, and probably indicates a compensatory activity of the spleen during a condition of impending dehydration.

The fact that there was a net decrease in plasma volume in most cases is again associated with the tendency to dehydration over the week's latent period.   However, in 6 cases there was an exceptionally large increase in plasma volume and 5 of these occurred during extremely hot weather.   One might postulate that *a*) the thirst mechanism overcame the anorexia in these cases, causing an influx of water into the blood from the gastrointestinal tract, and *b*) the condition of decreased capillary permeability prevented the simultaneous downward adjustment of this increased plasma volume, which would normally take place by the rapid movement of fluid out of capillaries in other regions.   One might be permitted to speculate on the possible application of these findings to clinical conditions where it is desirable to bring about a stationary, moderately large blood volume.   A large priming dose of vitamin D followed by the intravenous administration of nutrient fluids over the several days' latent period might achieve this end.

Disappearance Rate of T-1824 as an Indicator of Capillary Permeability

The validity of the method was investigated in two ways; first a study was made of the effect of a known capillary poison, histamine, on the dye disappearance rate, and, secondly, a quantitative study was made of the transfer of dye from the blood to the lymph in order to see if the rate of disappearance from the blood stream is a true indicator of the transfer of dye across the capillary barrier into the lymph.

Effect of histamine on the dye disappearance rate.   Histamine was administered intravenously during the course of nine experiments.   Six of these experiments were similar to the routine dye disappearance test and three were acute experiments involving the collection of lymph, as described in the next section.

It was found that the disappearance rate was increased several fold by histamine. The control values were 6 to 11 per cent for the six routine experiments, and 4 to 6 per cent for the three acute experiments.   The disappearance rates found with histamine were 18 to 29 per cent for the former, and 12 to 19 per cent for the latter. Table 2 shows these data.   Figures 2 and 3 illustrate curves of single typical experiments of the routine and acute types, respectively.

It should be pointed out that the disappearance rate measured during histamine administration, in all likelihood, does not fully reflect the true state.   If we assume that histamine brings about a considerable exit of dyed fluid from the blood, as was evidenced here by the copious and heavily dyed lymph flow seen in the histamine-lymph experiments, then it is apparent that the dye disappearance rate as measured could not reflect the full degree of increased leakage.   It is possible to make a partial correction for this by using the increased hematocrit as an index for the decrease in plasma volume, and thus to correct the measured dye concentrations by the factor by which the plasma volume was altered.   This procedure, of course, neglects the factor of splenic contraction which may contribute to the rise in hematocrit, but nevertheless, the resulting 'corrected' disappearance rates are probably more accurate than the uncorrected ones.   Table 2 includes the 'corrected' rates based on the hematocrit values, and these range from 29 to 54 per cent.

The entire group of 9 histamine experiments presented a mean value of 20 per cent uncorrected, and 40 per cent 'corrected' disappearance rates, to be compared with the control mean of 7 per cent (s.d. = 2.8%).

TABLE 2. EFFECT OF HISTAMINE ON DYE DISAPPEARANCE RATE

CASE	EXPERIMENTAL CONDITIONS	HEMATOCRIT 15^8–60^8	DYE DISAPPEAR. RATE	

Control Experiments

CASE	EXPERIMENTAL CONDITIONS	HEMATOCRIT	DYE DISAPPEAR. RATE
		%	%
15a	Routine determination, no anesthesia	51–50	11
15b	" " " "	48–47	9
15c	" " " "	48–48	6
16a	" " " "	49–47	11
16b	" " " "	51–50	7
16c	" " " "	50–46	11
20	Acute lymph experiment, anesthesia	43–45	5
21	" " " "	38–41	4
22	" " " "	55–57	6
23	" " " "	51–53	5
MEAN			7.5
s.d.			2.8

Histamine Experiments

CASE	EXPERIMENTAL CONDITIONS	HEMATOCRIT	DYE DISAPPEAR. RATE	
			A[1] %	B[1] %
15d	Routine determination, no anesthesia	52–57	18	33
15e	" " " "	53–60	18	38
15f	" " " "	53–59	20	37
16d	" " " "	50–59	27	49
16e	" " " "	52–62	29	53
16f	" " " "	56–68	23	54
17	Acute lymph experiment, anesthesia	49–52	19	31
18	" " " "	59–63	16	29
19	" " " "	54–63	12	38
MEAN			20	40

[1] A = uncorrected; B = corrected according to text.

Correlation of dye disappearance rate with transfer of dye from blood to lymph. Ten fasted dogs were anesthetized with sodium barbital, and lymph from the thoracic or cervical lymph duct, which drained continuously, was collected for analysis during a control period and at two 15-minute periods which averaged 15 and 60 minutes after dye injection. Routine blood samples were taken simultaneously for determining the dye disappearance rate. In three cases histamine was injected during the

.course of the experiment, and of the remaining 7 dogs two had received massive doses of vitamin D four months prior to the acute experiments.

The control dogs displayed a lymph dye concentration curve which rose very slowly over one hour to about one-fifth to one-third of the serum dye concentration. In the histamine experiments, the lymph became very blue and flowed copiously after the drug injection, reaching the plasma level of dye concentration by one hour (fig. 3).
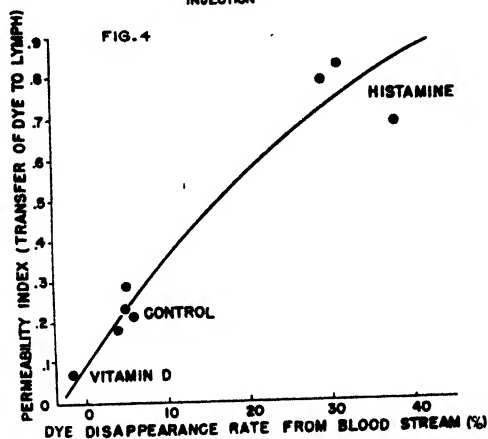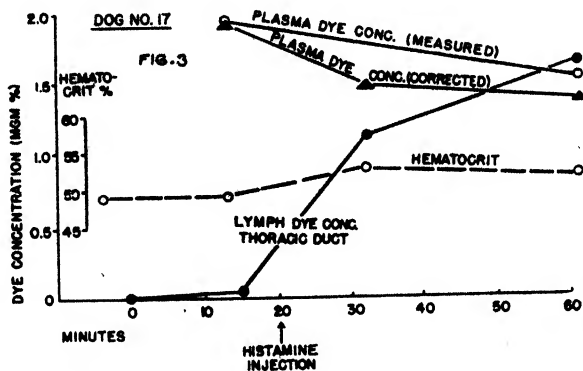
Fig. 3. ACUTE LYMPH EXPERIMENT on anesthetized dog showing effect of histamine on transfer of dye from blood to lymph.　Dye injected at o time.

Fig. 4. Relation between dye disappearance rate and transfer of dye from blood to thoracic lymph.

In order to make a quantitative correlation between dye disappearance from the blood and dye transfer to the lymph, it was necessary to use some numerical index of dye transfer, which would take into account the varying values of original plasma dye concentration.　The index used was the ratio of the increase in lymph dye concentration between 15 and 60 minutes after dye injection, over the 15-minute plasma dye concentration.　This ratio was calculated for each of the 10 lymph experiments, and the resulting figures, termed 'permeability indices' are shown in table 3, grouped according to conditions of theoretically increasing capillary permeability.　The dye

disappearance rates obtained simultaneously are also in this table. Two main points are brought out by these data. *a*) The figures for permeability index correspond qualitatively with the theoretical conditions of capillary permeability, from the decreasing effect of vitamin D, through the control range, to the increasing effect of histamine. *b*) In the case of the thoracic duct experiments, there is excellent correlation between the values for dye disappearance rate and the permeability indices obtained simultaneously. The correlation coefficient *r*, was found to be +0.956, indicating the validity of the dye disappearance rate test in detecting large differences in the conditions of permeability of the visceral capillaries. Figure 4 illustrates this point.

Discussion. The method used for testing capillary permeability seemed at first to be open to a number of theoretical objections. One was the rôle of phagocytosis

TABLE 3. PERMEABILITY INDEX UNDER VARIOUS EXPERIMENTAL CONDITIONS. CORRELATION WITH DYE DISAPPEARANCE RATE

CASE	LYMPH TESTED	EXPERIMENTAL CONDITIONS	PLASMA DYE CONCENTRATION		LYMPH DYE CONCENTRATION		PERMEABILITY INDEX $\frac{(D-C)}{A}$	DYE DISAPPEAR. RATE
			15′ (A)	60′ (B)	15′ (C)	60′ (D)		
			mg. %		*mg. %*			*%*
12	cervical	Vitamin D	1.20	1.08	0.00	0.00	.00	10
24	"	Control	1.93	1.75	0.00	0.08	.04	9
13	thoracic	Vitamin D	0.63	0.64	0.00	0.03	.05	— 2
21	"	Control	4.00	3.85	0.07	0.78	.18	4
22	"	"	1.54	1.45	0.03	0.34	.21	6
20	"	"	3.30	3.15	0.17	0.94	.23	5
23	"	"	0.93	0.88	0.09	0.36	.29	5
19	"	Histamine	2.21	1.37	0.15	1.68	.69	38
18	"	"	0.91	0.65	0.12	0.84	.79	29
17	"	"	1.88	1.30	0.03	1.60	.83	31

as an independent variable. That this is of negligible importance is implied by the case of the dog described in the preliminary paper, where a large difference in the factor of phagocytosis is an independent variable. That this is of negligible importance is implied by the case of the dog described in the preliminary paper, where a large difference in the factor of phagocytosis (the reticulo-endothelial system was blocked) had no effect on the dye disappearance rate. This might be expected on the grounds that during the first hour after dye injection, the dye particles are mainly concerned with being distributed into the lymph.

Another objection was the effect of a possible contraction of the spleen during the course of a determination. However, since the spleen delivers very cell-rich blood, this would not greatly affect concentration of plasma constituents, and, furthermore, the rise in hematocrit would constitute a check on the test.

A change in the circulatory conditions of the liver might be expected to affect the

dye disappearance rate, and it is probably this factor which causes the considerable degree of control variation. These normal variations, however, are not large enough to invalidate the method for detecting marked changes in capillary permeability such as caused by histamine and vitamin D.

The strongest argument in favor of the method is the excellent correlation which was found between the dye disappearance rate from the blood stream and the measured transfer of dye from blood to lymph. It should be pointed out that the concentrations of plasma dye used here were low, and that the situation might be different with higher blood concentrations, a fact which was suggested by later work in which the amounts of injected dye were four times as great.

Naturally, the method would not be valid under extreme conditions, such as acute trauma, where the integrity of the vascular system might be so grossly impaired as to permit frank hemorrhage or leakage of large amounts of practically whole blood into extravascular spaces. Under these conditions, the concentration of any one element within the waning vascular pool could remain quite unchanged.

SUMMARY AND CONCLUSIONS

After a latent period of five to eight days following a highly concentrated dose of vitamin D, the following effects were observed in dogs: *a*) a decreased dye disappearance rate in 76 per cent of the cases; *b*) weight loss of approximately 10 per cent, caused by lowered intake of food and water; *c*) an increased hematocrit in 100 per cent of the cases. This phenomenon was ascribed to splenic activity accompanying the state of dehydration; *d*) a relatively unchanged plasma and total blood volume.

The administration of histamine caused the dye to disappear from the blood and appear in the lymph at a rate approximately four times greater than the controls. The quantitative study of the transfer of dye from blood to lymph over a wide range of conditions of capillary permeability, demonstrated an excellent correlation between the dye disappearance rate from the blood stream and the appearance of dye in the thoracic lymph.

A highly concentrated dose of vitamin D decreases the disappearance rate of T-1824 from the blood stream of dogs, after a latent period of about one week. The disappearance rate of the dye T-1824 from the blood stream, for low concentration of plasma dye, is a good indicator of the transfer of dye from blood to lymph, and thus, inferentially, measures the general state of permeability of the visceral capillaries.

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SPECTROPHOTOMETRIC MEASUREMENT OF TRACES OF THE DYE T-1824 BY EXTRACTION WITH CELLOPHANE FROM BOTH BLOOD SERUM AND URINE OF NORMAL DOGS[1]

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DURING the past 60 years a number of diazo dyes of biological interest have been synthesized by coupling o-tolidine with sulfonated aminonaphthols like 1-amino-8-naphthol-2,4-disulfonic acid (1). The latter product, briefly called T-1824, has become widely known because of its suitability for estimating plasma volume (2–9).

Several years ago it was shown that T-1824 has marked affinity for serum albumin, much lesser affinities for alpha and beta globulins and no affinity for gamma globulin (9) or fibrinogen (5). It is probable that T-1824 in the bloodstream exists chiefly in the form of a dye·albumin compound (7, 9). As previously recognized, the extraction of T-1824 from a dye-tinged blood sample involves the splitting of dye·albumin (10). There is a simple way of liberating dye from albumin and this can be done by adding a detergent such as Aerosol OT (11). Dye, thus liberated, also has an affinity for cellophane (9, 11). It therefore seemed possible that traces of T-1824 in various biological fluids could be both extracted and selectively concentrated on small foils of cellophane. Then the amount of dye could be estimated colorimetrically.

It is the purpose of this study to indicate the procedures which can be used in the quantitative estimation of T-1824 by a method based on these principles. The method has been tested by the recovery of known amounts of T-1824 in 0.2 ml. of blood serum and in half-hour urine samples. Small, definite quantities of this protein-binding dye were found to pass from the blood into the urine of dogs. The T-1824 clearance proceeds at the same rate as that predictable for serum albumin.

GENERAL PROCEDURE

Two sizes of single thickness foils were cut from Cellophane dialyzer tubing.[2] A foil, about 1 x 2 cm. or 2 x 3 cm. in area, was suspended on a glass needle in order to hold the foil in a vertical position when immersed in fluids contained in a 50 ml. Erlenmeyer flask. The flask was covered with an inverted beaker and placed on a steam-coil water bath at 70° to 73°C. Test foils were soaked in saline, urine or diluted serum variously tinged with dye. Control foils were soaked in the same fluids except that dye was absent. The dyeing process was stopped at any desired point by transferring the foils to 0.9 per cent NaCl. Traces of adhering precipitate, when present, were removed by rinsing and if necessary by brushing.

The optical density of the dyed foil in comparison to its control was determined with a König-Martens visual spectrophotometer. Both foils were wet with water and pressed between two microscope slides clipped against a slotted vertical brass plate. A foil, thus prepared, gave identical optical densities when shifted from place to place before the light beam. Also the readings did not change with time. Determinations made on a number of dyed foils showed that the maximum optical density occurred at 635 mμ whereas that (4) for dye in serum is at 625 mμ and in either 0.9 per cent NaCl or water at 605 mμ. In consequence the optical densities were always compared at the wave length of maximum absorption. Since the foils were not of equal area, although apparently of identical thickness, it next was necessary to know their dry weights in order to determine the total amount of dye present. The small foils ranged from 5.2 to 6.8 mg. and the larger from 14.7 to 18.1 mg.; they were weighed to the nearest 0.1 mg.

Known solutions of T-1824 in a given concentration of NaCl were prepared by diluting a standardized ampule solution (0.48 per cent in water) with glass distilled water and the required volume of 9.0 per cent NaCl.[3] Unless otherwise indicated the dilutions were made so as to give final NaCl concentrations of 0.9 weight per cent. All glassware used was Pyrex, cleaned with chromic acid, rinsed, and steamed.

RESULTS

Sorption equilibrium. After 20 hours an equilibrium (fig. 1) occurred in the sorption of dye by a foil in the presence of 25 ml. of a 1/10,000 dilution of the ampuled dye. Equilibrium was indicated by the relative constancy of the *cellophane value, which here is defined as the product of the optical density at 635 mμ and the weight in mg. of any foil.* This dyeing process was quite striking to behold. The blue color of the fluid was very faint at the beginning, while at the end the foil was blue and the fluid was colorless. If all the dye had migrated to the foil, this would mean that more than a 5000 fold concentration step occurs in the transfer from the original dispersion volume.

In order to test for completion of sorption the foils were removed after 23 hours from three reaction flasks and a new foil was placed in each flask. Twenty-four hours later these second cellophane values were less than two per cent of the first ones. When for three other reactions the first 18-hour cellophane values were compared with their second values, the latter were less than six per cent of the former. These results together with those obtained from a 2/10,000 dilution, lead to the conclusion that at equilibrium at least 98 per cent of the dye combines with the foil.

Influence of electrolyte and volume on sorption changes. NaCl had a marked effect upon the equilibrium position. Sorption was nearly complete in 0.9 to 3.0 per cent NaCl, whereas it was incomplete in lesser concentrations of NaCl (fig. 1, insert). As previously observed (9) there apparently was no sorption from aqueous solutions. Water also removed dye previously sorbed from NaCl solutions. These facts were ascertained by the following additional means. A dyed foil with a cellophane value of 2.48 together with an undyed foil was placed in 25 ml. of glass distilled water at 70°C. Eighteen hours later both foils were colorless and the water had become tinged with blue.

The pH was changed in two separate dilutions of the same amount of dye by making each dilution 0.01 M with respect to either monobasic or dibasic phosphate buffer salt. The glass electrode readings corresponded to pH 4.6 and to pH 8.5.

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[3] The blue dye T-1824 was furnished by the Warner Institute for Therapeutic Research, New York City.

Triplicate cellophane values from these two solutions were identical with those from an unbuffered solution of the dye.

At equilibrium the cellophane values remained unchanged whether the volume was 25 ml. or had been increased by the addition of 75 ml. of 0.9 per cent NaCl. Therefore, it is concluded that sorption at 70°C. is relatively uninfluenced by these changes in pH or volume, provided the NaCl concentration is at least 0.9 per cent.

Foil size, cellophane value and amount of T-1824. A small foil (5 to 7 mg.) was placed in 25 ml. of any one of six different concentrations of T-1824. After equilibrium time at 70°C. the cellophane value was estimated. The means for triplicate or quadruplicate determinations, thus obtained, are plotted in figure 2. It may be seen that in less than 2/10,000 dilutions of ampuled dye a direct proportionality was found between the amount of dye originally in solution and the cellophane value
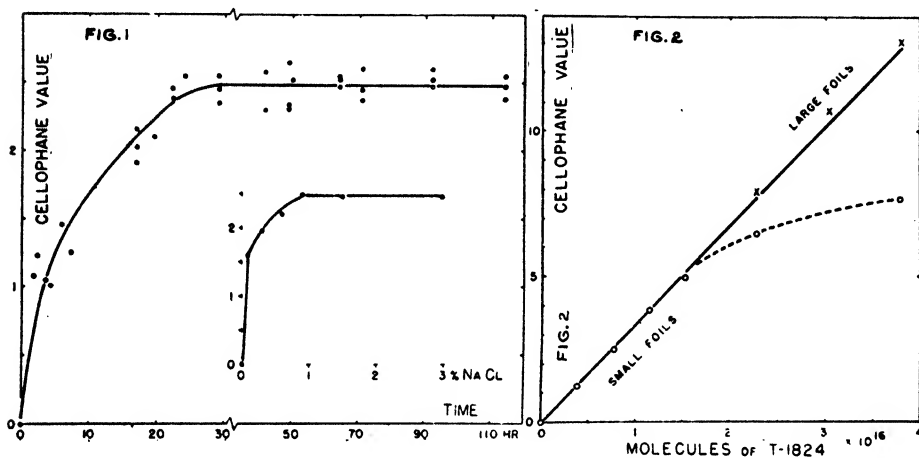
Fig. 1. SORPTION OF T-1824 DYE by cellophane as a function of time from 25 ml. of 1/10,000 dilution of 0.48 % T-1824 in 0.9 wt. % NaCl. Temperature is 70° to 73°C. *Insert:* promotion of sorption equilibrium by NaCl. Both ordinates express units of cellophane value = optical density at 635 mμ × wt. mg. of foil 0.00186 cm thick.

Fig. 2. RELATION BETWEEN FOIL AREA, cellophane value and amount of T-1824. Dilution range is 0 to 5 parts in 10,000 of 0.48 % T-1824 in 0.9 wt. % NaCl.

developed at equilibrium. Beyond this concentration the direct proportionality ceased to exist as indicated by the departure from linearity (dotted line, fig. 2), at 3/10,000 and 5/10,000 dilutions and also by the visible retention of dye in the latter solution.

When larger foils (15 to 18 mg.) were used the cellophane values from 3/10,000, 4/10,000 and 5/10,000 dilutions of T-1824 returned to the same proportionality obtained for the small foils in the lower concentrations of dye (*solid line*, fig. 2.). Evidently an excess of cellophane must be present in order to demonstrate a simple, linear relationship between the amount of dye and the cellophane value. In 25 determinations the two greatest deviations from proportionality were less than ±8 per cent. Since unknown variations in dyeing and known variations in instrumenta-

tion could account for the observed deviation, it is concluded that this direct relationship is an index of the amount of T-1824.

It is possible to relate the cellophane value of a dyed foil to the number of molecules of T-1824. If in these solutions the dye is a monomer, the molecular weight is 960 (1) and the calculated number of molecules in 25 ml. of a 1/10,000 dilution is 0.76×10^{16}. Thus, according to the data of figure 2, a cellophane value of 1.00 represents 0.30×10^{16} molecules of T-1824.

The above cellophane values for T-1824 were obtained with foils cut from the same piece of cellophane. This material was 0.00186 cm. in thickness. For the sake of future comparison with foils of a different thickness but identical chemical constitution, it is necessary to state the molar absorption coefficient, ϵ, for the dye cello-
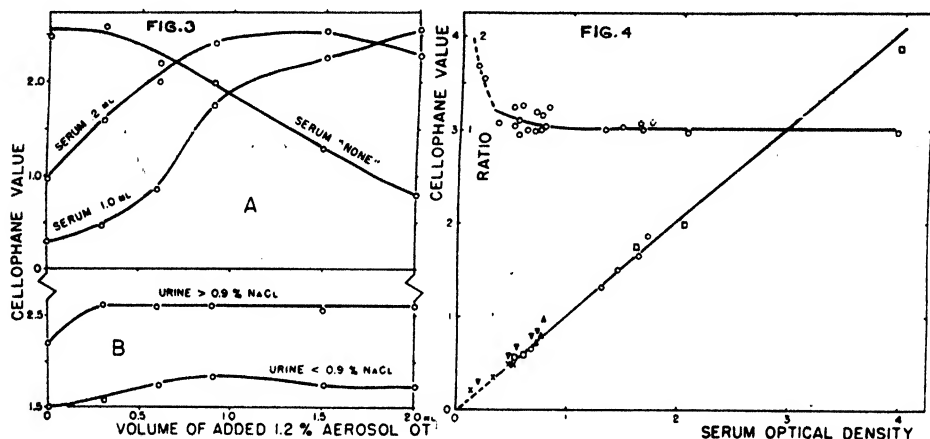
FIG. 3. EFFECT OF AEROSOL OT on sorption and recovery of T-1824 from dog blood serum and urine. A cellophane value of 2.50 corresponds to recovery of the 7.6×10^{15} molecules or 12.2 μg. of T-1824 originally dispersed in the diluted blood serum or urine. *A:* when 0, 0.2 or 1.0 ml. of blood serum is first mixed with 25 ml. of 1/10,000 dilution of 0.48 % T-1824 in 0.9 wt. % NaCl. *B:* when NaCl is either added or not added to 25 ml. of 1/10,000 dilution of 0.48 per cent T-1824 in urine of water diuresis.

FIG. 4. COMPARISON OF CELLOPHANE VALUE with serum optical density (in 0.5 cm. cuvettes) for successive blood samples from 5 dogs.

phane. This coefficient was calculated from the standard equation, $\epsilon = D/c.d$, expressing both dispersion volume and thickness in terms of mass and specific gravity. The latter cancels out and the final equation becomes: $\epsilon = K \cdot DM/N$, where DM is the cellophane value, N the number of molecules in the solution and K, a constant depending on foil thickness and equilibrium position, is 1.99×10^{20}. Fifty-one determinations, illustrated in figures 1 and 2, yield a mean of 66,300 and standard error of ±3,700. This coefficient is characteristic of dye·cellophane. It is smaller than that of dye·albumin. The molar absorption coefficient of T-1824[4] in either blood plasma or serum is 77,800. This value increases in 0.9 per cent NaCl to 85,200 and in water to 93,800.

Liberation of dye from diluted serum by Aerosol OT.[5] When blood serum containing 5.4 per cent protein (refractometric analysis) was mixed with solutions of T-1824, less dye was available for sorption by cellophane. This effect was qualitatively observed by Rawson (9) and was quantitatively verified at a higher temperature in the course of the present study. The values on the ordinate of figure 3a show that the addition of 0.2 ml. of serum to 25 ml. of a 1/10,000 dilution of 0.48 per cent T-1824 reduced the cellophane value from 2.42 to 0.96 and with 1.0 ml. of serum the value fell to 0.30. Dye was not sorbed from undiluted serum.

Aerosol OT altered the foregoing relationship among the components of this dye-protein-cellophane system (11). When sufficient Aerosol OT was added at room temperature to either the mixture of dye and serum or to only the serum solution, a faint turbidity immediately appeared. However, there was no turbidity in the absence of serum. The Aerosol OT evidently freed dye from protein as indicated by the upward course of the two serum curves in figure 3a. When 0.9 ml. of the Aerosol OT solution was added to the mixture of 25 ml. of T-1824 solution and 0.2 ml. of serum, an equilibrium cellophane value of 2.38 was attained. Approximately all of the 7.6×10^{15} molecules of T-1824 were therefore combining with the cellophane. The data also show that a greater amount of Aerosol OT was needed to free the dye from 1.0 ml. of serum. In the absence of serum (fig. 3a, serum none) there was a decrease in sorption of dye with the addition of Aerosol OT. Whether in the latter system the Aerosol OT competes for T-1824 or for cellophane is unknown. However, the arrangment of this family of curves (fig. 3a) provides a means of testing for protein. The rising curve shows the presence, whereas the falling curve must indicate the absence of dye-binding protein.

Previous observations together with those noted above suggest that this liberation of dye from serum proceeds through complex reaction steps involving several features of interest. Reagents, such as trichloracetic acid, precipitate the usual dye-tinged serum leaving all of the dye still combined with the insoluble proteins (6). This sort of combination has recently been measured in various mixtures of T-1824 and serum albumin or globulins (12) at pH 2.5. In contrast to the usual protein precipitants various synthetic detergents have properties of interest to recovery of T-1824 from serum proteins. There are many known reactions between various proteins and polar-nonpolar anions of different'chemical structure containing more than 10 carbon atoms. Aerosol OT in sufficient amount was early found to denature beef hemoglobin (13). Mixtures of horse serum albumin and sodium dodecyl sulfate combine in characteristic fashions in relation to pH and temperature (14). It is therefore probable that Aerosol OT reacts with the serum proteins and in so doing combines with at least those groups available for attachment of T-1824, thus freeing dye for sorption on cellophane. This competition between Aerosol OT and T-1824 is similar to that observed by Klotz in which a number of different organic anions compete with sulfonated anions such as azosulfathiazole for the ε-ammonium of lysine in bovine serum albumin (15).

Estimation of dye in blood serum. Dye-tinged serum was obtained from 5 dogs none of which had ever received T-1824. A control blood sample was taken. Then

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[5] The dioctyl sodium sulfosuccinate described in Aerosol Wetting Agents, 1946, American Cyanamid Co.

a measured volume of o.48 per cent T-1824 solution was injected into a jugular vein. After 15 minutes and at intervals during the next four to six hours, dye-tinged blood was withdrawn from the opposite jugular vein. A blood sample was also taken on the following day.

Direct spectrophotometric determination of optical density at 624 mμ was made on each of these 22 samples using o.5 cm. cuvettes. One of the samples was so dense that it was necessary to make a two-fold dilution with o.9 per cent NaCl in order to measure its optical density.

Triplicate cellophane determinations were also made on each of the samples. To 25 ml. of o.9 per cent NaCl was added o.2 ml. of dye-tinged serum and 1.0 ml. of Aerosol OT solution (1.2 per cent in water). After one-half hour at room temperature a small foil was added, and the flask was covered and placed at 70°C. for 24 to 30 hours. A control foil was similarly exposed to the serum obtained before injection of dye. The means for these cellophane values are indicated along the ordinate in figure 4.

Since the reaction between dye·protein and Aerosol OT releases all of the dye (fig. 3a), it was not surprising to have found the linear relationship which intersects the origin in figure 4. When the serum was deeply tinged with dye, the cellophane value from o.2 ml. was only 1.02 times greater than the serum optical density. With serum optical densities between 1.0 and o.3, the cellophane value increased to 1.10 times as indicated by the slight upward trend of the ratio curve (fig. 4). Two of the 24-hour samples, which were less than o.3 optical densities, gave unusually high cellophane values thus causing the ratio to approach 1.7:1.0 and perhaps marking a lower limit where instrumental error makes it difficult to secure accuracy with only o.2 ml. of serum. The near identity of the cellophane value and the serum optical density occurred only through the chance that the chosen volume of serum contained the quantity of dye which gave these cellophane values on foils of this particular thickness. For example, doubling either the foil thickness or the volume of dye-tinged serum yielded cellophane values which were approximately twice the values for directly measured serum optical densities.

Recovery of T-1824 from urine. A solution was prepared by diluting 25 ml. of a 1/1,000 dilution of o.48 per cent T-1824 to 250 ml. with dog urine. The urine had been obtained by catheter in the course of a diuresis following the introduction by stomach tube of tap water in the amount 80 ml/kg. of body weight.

The equilibrium cellophane value from 25 ml. of this solution of dye in urine was 1.50 (fig. 3b) and was never greater than 1.85 when Aerosol OT was added. However, the addition of 2.5 ml. of 9 per cent NaCl together with Aerosol OT yielded cellophane values as great as 2.60, which represents complete recovery of dye. The dyeing process was promoted by NaCl but at least o.3 ml. of 1.2 per cent Aerosol OT was also needed for complete sorption. This, together with the fact that sorption did not decrease with more Aerosol OT, showed that there was binding of both T-1824 and Aerosol OT by urine constituents.

Excretion of T-1824 in the urine. A typical experiment was performed using a 12.5 kg. dog which had never received any T-1824. Tap water, o.7 l., was introduced by stomach tube. A urinary catheter was inserted, and an one-hour urine sample

together with the usual 10 ml. of 0.9 per cent NaCl rinse was collected before the taking of control blood and the injecting of 6.5 ml. of 0.48 per cent T-1824. During the next five hours urine was collected at half-hour periods. Then the animal was turned loose. Shortly before the eighth hour 0.3 l. of water was given and an one hour urine sample was obtained. This routine was repeated for the 12th-, 24th-, and 75th-hour urine samples. Blood samples were taken at different times. The time-concentration curve of T-1824 in the plasma (5) is shown in figure 5.

To each of the urine samples, thus collected, was added one ninth its volume of 9 per cent NaCl, 1.0 ml. of 1.2 per cent Aerosol OT and one of the small-size cellophane foils. After 30 hours at 70°C. the foil was removed, and its cellophane value
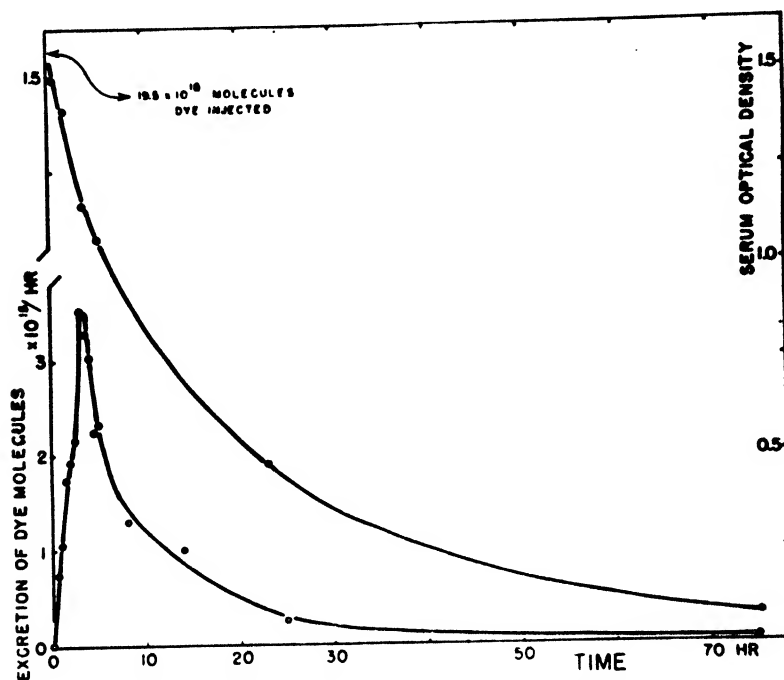
Fig. 5. RATE OF EXCRETION and serum optical density of T-1824 (in 0.5 cm. cuvettes).

was estimated from the optical density and weight. The amount of T-1824 in any urine sample was found by the correspondence of unit cellophane value to 3.0×10^{15} molecules of T-1824. The hourly rates of excretion are indicated by the lower curve, which rises for $3\frac{1}{2}$ hours and then falls (fig. 5).

Very little dye was excreted (table 1). In the first day only 0.15 per cent of the amount injected appeared in the urine. This value fell during the next two days to only 0.04 per cent. Most of the dye which disappeared from the blood obviously left by routes or mechanisms other than the kidneys (7, 12, 16). Nevertheless, renal excretion of dye was related to the plasma concentration. At any moment the amount gained by the urine was about 0.2 per cent of the amount lost from the circulation. Although the plasma concentration decreased to 30 per cent (fig. 5), the

renal clearance of T-1824 remained at 1.4 or 1.2 μl. per minute throughout the first day following injection of dye.

At the concentrations used for estimation of plasma volume, T-1824 has not previously been found in urine unless abnormal amounts of protein were also excreted (2, 7, 17). The cellophane test shows that dye is also excreted by the normal kidney but in such minute amounts that the visible presence of dye is masked by the usual color of urine. Since preliminary qualitative tests on 6 dogs showed that dye was excreted following the intravenous injection of T-1824, quantitative studies were made on 8 additional dogs, 4 of which had water diuresis. The results show that similar renal clearances occurred irrespective of sex, weight or urine flow (table 2).

TABLE 1. EXCRETION OF T-1824 BY THE KIDNEY DURING PERIODIC WATER DIURESIS IN DOG *8* INITIAL PLASMA VOLUME IS 610 ML.

TIME AFTER INJECTION	MOLECULES T-1824 EXCRETED $\times 10^{-15}$	PERCENTAGE OF AMOUNT INJECTED	PERCENTAGE OF AMOUNT LOST FROM PLASMA	RENAL CLEARANCE
hr.				$\mu l/min.$
0.5– 3.5	7.0	0.04	0.18	1.4
3.5–24	21.3	0.11	0.24	1.2
24 –75	7.9	0.04	0.18	0.7

TABLE 2. RENAL CLEARANCE OF T-1824 IN INDIVIDUAL DOGS ARRANGED IN ORDER OF AVERAGE URINE FLOW

DOG	SEX	WT.	TIME AFTER INJECTION	URINE FLOW	CLEARANCE
		kg.	hr.	ml/min.	$\mu l/min.$
1	Female	10.2	0.5–4.5	0.04	1.7
2	Female	9.4	0 –8	0.05	2.0
3	Male	9.0	0.5–8	0.08	1.9
4	Male	10.9	1 –6	0.09	1.4
5	Female	11.0	0.5–4.5	0.98	2.2
6	Male	13.0	0 –6	1.03	2.1
7	Male	10.5	0 –6.5	1.04	1.4
8	Male	12.5	0.5–3.5	1.37	1.4

Höber's studies offer reasons for suspecting that T-1824 is not secreted. Since disulfonated dyes with symmetrically distributed sulfonic acid groups are not actively transferred from solution in the Ringer-perfused renal portal system of a frog (18), it might be inferred that the tetrasulfonated T-1824 dye would have escaped only by passive transfer. This inference together with the observed clearances suggests that T-1824 passes into capsular fluid in the course of glomerular filtration. After being filtered, the amount of T-1824 collected in the urine is independent of variations in tubular reabsorption of water.

T-1824 in the amounts injected for determination of plasma volume probably is present in the blood stream only as dye-albumin (7, 9). Hence, it was decided to compare the T-1824 clearance with that for protein calculated from data found by a sensitive physical method. The clearance of T-1824 and of albumin should be

identical. The surface film area technique has recently given evidence for a mean concentration of 3.7 mg. per cent of protein in urine of normal human males (19). Gunton and Burton do not speculate on the route of protein excretion, but if the urine protein of their subjects had been albumin, the plasma albumin 3.5 per cent, and urine flow one ml. per minute, then the albumin clearance should be one μl. per minute. This clearance is of the same order of magnitude as that for T-1824 in the dog.

DISCUSSION

A method has been devised for the extraction and estimation of amounts of T-1824 as small as 0.5 μg. The dye is sorbed on a cellophane foil and measured with a spectrophotometer. The principle of the method could be described as being an extension of the Detergency Triangle of McBain (20). The extension to the T-1824 system may ideally be given by the following equation:

$$\text{protein} \cdot \text{dye} + \text{soap} + \text{cellophane} = \text{protein} \cdot \text{soap} + \text{cellophane} \cdot \text{dye}.$$

Although there is a strong affinity of dye for protein, advantage may be taken of the affinity of T-1824 for cellulose in order to extract this dye from blood serum or urine. The amount of soap needed for the desired ion exchange was found by mixing various amounts of Aerosol OT with protein·dye and testing for sorption of dye on excess cellophane. With cellophane foils of identical area and thickness the optical density at 635 mμ alone was an index of dye sorption. Since it was neither convenient nor accurate to cut all of the foils to the same area, the T-1824 was measured by the product of the optical density and the dry weight of a foil which was 0.00186 cm. thick. This product is called the cellophane value for T-1824 and is proportional to the amount of dye present in the original dispersion volume. For example, a cellophane value of 1.00 corresponds to 3.0×10^{15} molecules or 4.8 μg. of T-1824.

Many of the factors which influence the sorption of T-1824 are recognized to be of importance to the physical chemistry of dyeing. A survey of this subject (21) lists dyes which are similar in their behavior to that of T-1824. The characteristics of various textile dyes have been quantitatively studied by means of their sorption on regenerated cellulose films. Diffusion within cellulose itself is believed to be the slowest reaction step encountered in the entire dyeing process. Finally, through comparison with the dyes observed by Valko (21), it could be expected that the anionic dye T-1824 would be dispersed as a monomeric anion in 0.9 per cent NaCl at 40° or 70°C. In this connection the present report describes studies on the sorption of T-1824 as affected by temperature, dye concentration, NaCl, pH, Aerosol OT and serum or urine constituents. Consequently, the conditions may be stated under which dye can be extracted from blood or urine as completely as it can be from NaCl solutions. In the presence of excess cellophane the sorption of T-1824 is at least 98 per cent complete after more than 20 hours at 70°C. in NaCl of 0.9 to 3.0 per cent. Sorption is unaffected by changes in dispersion volume from 25 to 100 ml. and in pH from 4.6 to 8.5. However, sorption does depend on the availability of free T-1824 and hence on the absence of free serum protein or excess of free Aerosol OT. If 1.0 ml. of 1.2 per cent Aerosol OT is added to a mixture of 0.2 ml. of dye-tinged dog blood serum and 25 ml. of 0.9 per cent NaCl maximum sorption occurs. The fact that Aerosol OT was needed for complete liberation of T-1824 from diluted serum (figs.

3a and 4) lends support to the view that this dye is strongly bound to albumin (7, 9) and that the relatively slow disappearance of T-1824 from the bloodstream is a function of its marked affinity for serum albumin (9). It is necessary to make dog urine at least 0.9 per cent with respect to NaCl and to add at least 0.3 ml. of the Aerosol OT solution in order to get maximum sorption from half-hour urine samples.

In the course of the present studies it was noted that there was no change in the optical density at 635 mμ of the control foils. Evidently the exposure to dye-free urine or blood serum did not result in sorption of urine or serum constituents with chromophoric groups like those of T-1824. This observation indicates that further work may discover a practical advantage in use of this cellophane method to overcome the difficulties encountered (8) when lipemia or varying hemolysis is present, both of which interfere with direct spectrophotometric estimation of T-1824 in the serial blood samples used for measurement of plasma volume. Since the cellophane value of T-1824 from 0.2 ml. of serum is only 1.10 to 1.02 times greater than the serum optical density, it can be corrected and substituted into the equation (8) used in calculating the plasma volume.

The rate of arrival of T-1824 in the urine with respect to the T-1824 concentration in dog blood yields a plasma clearance of 1 to 2 μl. per minute. By assuming this clearance value to be entirely a function of renal activity, it then becomes of interest to inquire into whether T-1824 leaves the blood in the form of free dye, as a dye·albumin compound or as a mixture of the former and latter. This inquiry will consider the evidence for the binding of dye in blood serum and also in urine. For the purpose of measuring the plasma volume the initial plasma concentration of T-1824 is approximately 0.002 per cent (8). As revealed by electrophoretic analysis a 0.004 per cent concentration of dye in four-times diluted blood serum at pH 7.4 is entirely bound by serum albumin (9). If the dye concentration is increased to 0.098 per cent it then combines with alpha and beta globulins as well as with albumin. It is known that ionized basic groups in one mole of albumin bind somewhat less than 14 moles of T-1824 (9), but the dissociation constant for the dye·albumin at pH 7.4 is unknown. LeVeen and Fishman find that T-1824·albumin at pH 2.5 has an apparent dissociation constant of $K = 2 \times 10^{-6}$ and that at this low pH one mole of albumin binds at least 70 moles of dye (12).[6] There are several reasons for believing that K is less than 2×10^{-6}: it has been calculated for molarities existing before dilution and precipitation with trichloracetic acid solution; competition between anions of trichloracetic acid and T-1824 has not been considered; at the low pH the sulfonic acid groups of T-1824 are not entirely ionized (10), i.e. their pK $>> 1$ and < 2.5. For lack of information it is impossible to discuss the effect of electrostatic repulsion between T-1824 anions and T-1824·albumin as has been done in both fact and theory for azosulfathiazole (22). However, the value $K = 2 \times 10^{-6}$ suggests that T-1824 has an affinity for serum albumin which is several hundred times that of azosulfathiazole. The actual dye·albumin formed in blood is probably so weakly dissociated that the arrival of dye in the urine is an index of albumin excretion. Urine albumin is very dilute and must certainly be affected by urine constituents, yet it was necessary

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[6] In reply to a question in a letter from one of the authors a letter from H. H. LeVeen in part indicates that their published value is in error, should be changed from 2×10^{-3} to 2×10^{-6}, and this change will be requested of the editors.

to add Aerosol OT to dog urine in order to liberate dye for more complete extraction by cellophane.

SUMMARY AND CONCLUSIONS

The blue tolidine dye T-1824 has been quantitatively extracted from 0.9 to 3.0 per cent NaCl, blood serum and urine. Complete extraction, involving a great concentration step, is approached in the presence of excess cellophane after 24 hours at 70°C. Sorption is independent of certain pH or dispersion volume changes. The resulting dye·cellophane compound has a maximum light absorption at 635 mμ where the molar absorption coefficient is 66,300. The amount of sorbed dye was estimated in terms of the product of the optical densities at 635 mμ and the dry weights in mg. of foils which were .00186 cm. thick. This product, called the cellophane value for T-1824, at unity corresponds to 3.0×10^{15} molecules or 4.8 μg. of T-1824. Amounts as small as 0.5 μg. of T-1824 have been estimated.

Twenty-six determinations with a mean cellophane value of 2.50 had a standard error of 0.14.

In order to liberate dye for sorption by cellophane it is necessary to add the anionic detergent, Aerosol OT, both to diluted blood serum and to urine.

At the dye concentrations used for estimation of plasma volume the cellophane value with foils of this thickness for T-1824 from 0.2 ml. of dog blood serum is nearly equal to the serum optical density at 624 mμ in 0.5 cm. cuvettes.

A discussion of the occurrence of T-1824 in dog urine following its intravenous injection is presented with respect to the possibility that the T-1824 and naturally occurring albumin clearances are identical.

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SHOCK DUE TO ELECTRICAL INJURY IN FROGS

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IN THE PAST, study of the complex and confusing problems of shock have been left almost entirely to the clinical physiologist and the pathologist, and yet, apparently, there are aspects of the problem which can be attacked from the standpoint of general or cellular physiology. This work is part of a larger plan to study the pathogenesis of injury shock from the standpoint of cellular physiology.

If shock is produced largely as a result of the formation of toxic substance by injured tissues (and this is now generally admitted by those who follow the literature), then we should seek to discover why and how the injured cells produce toxic substances, what sort of substances are involved, and in what manner they produce their effect on cells in other parts of the animal.

For many years, one of us (Heilbrunn) has maintained that when cells are stimulated or injured, a protoplasmic change similar to blood clotting occurs (1, 2). It was postulated that this protoplasmic clotting produced a thrombin-like (or, perhaps, a thromboplastic) substance which could induce clotting in cells distant from those injured. When the protoplasm of a muscle is clotted, this results in contraction (3). Hence, one could easily assume that injured cells give off substances which would pass through the circulation to smooth muscle cells at some distance and that these cells would undergo violent contraction. The evidence in favor of such a point of view is abundant and many-sided, and some of it has been summarized in an earlier paper (4).

The purpose of this paper is to further test the above hypothesis. If injury to tissues produces injury substances which have an effect on protoplasmic clotting, presumably these same substances would also affect the clotting time of the blood. Accordingly, we attempted to produce injury and then study blood-clotting times in the injured animal.

MATERIAL AND METHODS

Frogs, *Rana pipiens*, weighing 17 to 23 gm. and of both sexes, were chosen as the experimental animal. In all, over 240 animals were used (including 40 controls).

Electrical stimulation was provided with an Ene-Volt, a variable transformer type of stimulating apparatus manufactured by Gorrell and Gorrell. Electrodes used in the muscle experiments were either copper wires or small iron plates measuring 1 x 2 cm. The current used had an E.M.F. varying between 10 and 400 volts and was applied for periods of one second to 20 minutes. The best results were obtained by using four pairs of plate electrodes fastened to the legs so that the thigh and calf

of each leg would be shocked simultaneously. An E.M.F. of 300 volts was used with this method, and shocks of one-second duration were given one or two times per minute. The current intensity with this method was 800 milliamperes through the entire system. The maximum temperature developed, measured by a fine wire thermocouple, was 33°C. in the thigh and 34°C. in the calf of the leg.

In the brain injury experiments, the electrodes were made of narrow sheet-iron strips painted with Cenco cement (a modified de Kotinsky cement), which is a non-conductor. The contact areas were 2.5 x 3.0 mm. One electrode was placed in the midline of the skull, over a spot between the orbits and the auditory plaques. The other electrode was placed in the midline of the roof of the mouth immediately anterior to the downward bulging of the orbits. The best results were obtained by using an E.M.F. of 100 volts and giving 10 shocks of one-second duration at the rate of one shock per minute. The current intensity delivered was 160 milliamperes. The maximum temperature reached was 34°C.

The blood-clotting time was determined by the capillary method. The heart was exposed through an opening in the upper abdomen and lower thorax. Blood samples were obtained by puncturing the ventricle with a capillary tube drawn out to a diameter of approximately .25 mm. The small end was cut off by scratching with a sharp piece of carborundum. This is an important detail because the end thus cut penetrates readily into the ventricle, promoting speed and minimizing the chance of error. Contamination of the blood sample by pericardial or tissue fluids produces error by shortening the clotting time. This was avoided by cutting away the anterior portion of the pericardial sac and blotting the heart with filter paper. We followed as a standard rule: never to take a blood sample from a visibly moist ventricle. Care was taken, as far as possible, to make each puncture through a fresh, unbroken portion of the ventricle. In the serial determination two samples were taken in immediate succession and the average value determined for each pair. In both the experimental and control animals used in serial determination, the relations of blood loss due to sampling were as follows: in each individual puncture approximately .0050 to .010 cc. of blood was withdrawn. The average amount lost through bleeding at each puncture was .010 cc. Most of the animals were sampled six to eight times (12–16 punctures) and thus had a total blood loss of 0.21 to 0.28 cc., or 16 to 20 per cent of the estimated total blood volume. The animals were neither pithed nor anesthetized.

<div align="center">OBSERVATIONS</div>

Local and distant effects. In the muscle injury experiments the lower current intensities and brief shocking procedures produced only a temporary coma within 45 to 90 minutes after injury. The higher dosages produced irreversible effects. With the copper wire electrodes an E.M.F. of 75 volts for 4 minutes produced irreversible depression and coma in 30 to 60 minutes.

When a single pair of plate electrodes was used, death was caused by an E.M.F. of 200 volts applied for 7 seconds to each thigh and calf, as follows: 3 shocks of two seconds duration spaced approximately one minute apart followed by a 4th shock of one-second duration. When four pairs of electrodes were used, shocking all parts of the legs simultaneously, the E.M.F. was 300 volts.

The local effects were characterized by *hyperemia* of the skin beneath and be-

tween the electrodes and *marked swelling and rigor* of the hind legs, at first between the opposite electrodes.   Later the swelling involved the entire legs, and the rigor became somewhat relaxed.

The general effects consisted of a slight lethargy, which disappeared in 3 to 5 minutes after injury.   In about 20 minutes lethargy returned; and sooner or later, stupor developed.   The front legs became sluggish.   With this there was an apparent loss of initiative, which was accompanied by loss of the corneal reflex.   During this stage the animal was still responsive to mechanical stimuli.   In about 30 minutes to one hour came the stage of marked depression, during which no movement at all or only the very slightest movement of the front legs could be elicited by prodding.

The effects on the heart varied in degree.   In the experiments with wire electrodes the heart continued to beat for 12 to 24 hours after the onset of coma.   In the other experiments with plate electrodes, the heart stopped usually within $2\frac{1}{2}$ to $3\frac{1}{2}$ hours after the onset of depression.

TABLE 1.   EFFECTS OF ALTERNATE REMOVAL AND REAPPLICATION OF LIGATURES TO THE LEGS OF A FROG GIVEN ELECTRICAL INJURY

TIME	PROCEDURE	REACTION
min.		
0	3 female frogs shocked on each thigh and calf of the leg, then ligatures were applied just below the inguinal region	All frogs remained briskly reactive and normal, except for loss of use of hind legs
60	Left leg of 1 frog freed of ligature	Depression observed within 10 minutes. Loss of use of front legs
85	Left leg retied	Within 20 minutes brisk reactivity observed
150	Right leg freed of ligature	Depression returned within 10 minutes
165	Right leg retied	Within 10 minutes, some return of reactivity

The 2 remaining frogs (with ligatured legs) remained briskly reactive for at least 6 hours.

In brain injury the toxic effects were variable.   Coma beginning with the first shock and lasting from 15 minutes to $2\frac{1}{2}$ hours was observed in nearly all the animals. The animals which recovered lived 16 hours to three days.   The animals receiving 80 or 100 volts had irreversible coma.

Effects of interrupting the circulation.   The circulation was interrupted either by excision of the heart or ligating the thighs immediately before inflicting injury.   In all cases the depressant effects of electrical injury were confined to the hind legs. The entire upper part of the body remained normally reactive for two to three hours in the 5 frogs whose hearts had been removed, and for 1-2 days in those whose legs were ligated.   Ten controls showed the usual reaction of profound depression within the expected time of 30 to 60 minutes.   Later, 5 frogs with ligatures were shocked and after 30 minutes the ligatures were removed and profound depression and coma followed in a manner similar to that previously described.   Later, 3 frogs were given a lethal dose of electrical injury to the hind legs.   The ligatures were alternately removed and reapplied.   The observations are recorded in table 1.

Changes of the coagulation time of the blood.   Experiments to determine the effects

on blood coagulability were done by giving the animals a lethal dose of electrical injury and taking the clotting time at various intervals afterwards.

Ten animals were shocked.   In 2½ hours 5 of the animals were opened.   In 2 of the animals the clotting time was indefinite; in the remaining 3 the values were 12, 30 and 30 minutes (av. 12 minutes).   After 18 hours the remaining 5 showed a range
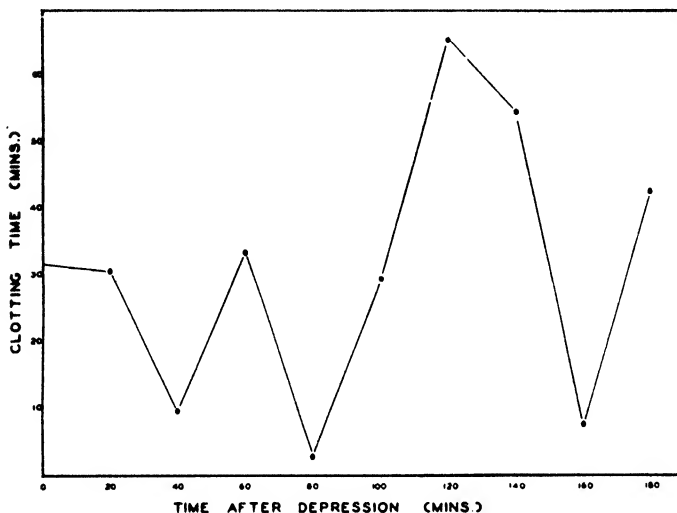
Fig. 1. EFFECT OF SHOCK due to electrical injury on the blood clotting time of frogs. Each point represents the average value obtained from the clotting time of 5 frogs at intervals after the onset of visible depression.
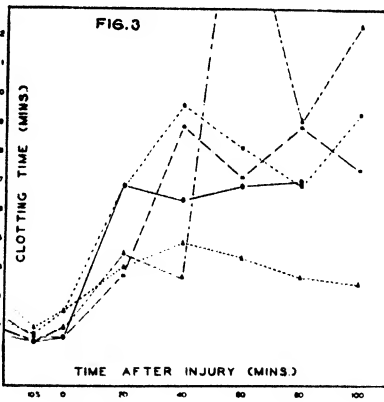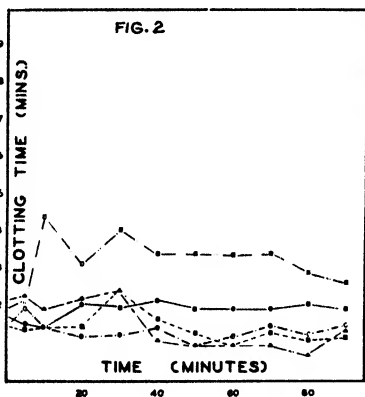
Figs. 2 and 3. COMPARISON OF BLOOD-CLOTTING CHANGES of control frogs (fig. 2) with those of frogs after receiving electrical injury of muscle (fig. 3).   Each curve represents the changes in the clotting time of one frog.   In fig. 3, samples were taken before shocking, after the 10th shock (10S), the 20th shock (o) and every 20 minutes.

of 4 to 17 minutes (av. 8 minutes).   Fifteen untreated frogs, used as controls, showed a range of 1.5 to 4.5 minutes (av. 2.4 minutes).

A more complete picture was obtained by shocking 50 frogs and averaging the clotting time in groups of 5 frogs at each of the following intervals: at the beginning of coma; then at 20-minute intervals thereafter for three hours.   Ten controls were

used, and their average clotting time was three minutes.   Results of the experiments are shown in figure 1.   Indefinite clotting times were given an arbitrary value of two hours, for the purpose of averaging.

Later, serial determinations were made on 10 frogs.   The results were shown in figures 2 and 4.   Five untreated frogs were used as controls.   The results are shown in figures 3 and 4.   Figures 2 and 3 should be compared, as they show the changes of the clotting time of each animal and indicate the extent of individual variations.

In the brain injury experiments (fig. 4), 10 animals were used.   The effects of electrical injury on the clotting time were substantially the same as in the muscle experiments.

Fig. 4. EFFECT OF ELECTRICAL INJURY on the clotting time of frogs. Comparison of averages obtained from test animals and controls.   The asterisks (*) represent points where the clotting time became indefinite.   The curves represent only the finite values. KEY: Controls, △ - - - - - △; muscle injury, O—··—O; ●——●.
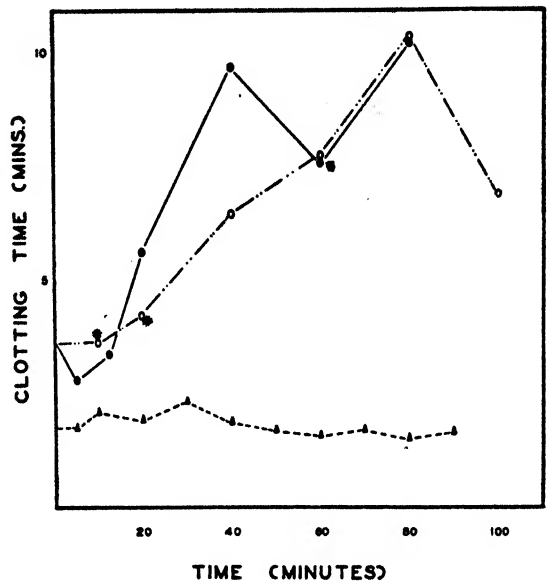
DISCUSSION

The results which have been stated give an affirmative answer to the three questions which were put at the beginning of our experiments: *Profound systemic physiological changes can be brought about in an animal by means of electrical injury to one part of the body.*   This statement is in agreement with previous work done in this laboratory on other types of injury.   The literature on electropathology is prodigious, but it deals chiefly with pathology caused by industrial accidents, criminal electrocution and accidental electrocutions from house currents, all of which involve excessive voltage and amperage and, often, long periods of contact with the production of high temperatures within the tissues.   Another trend of recent years in the study of electropathology has been in the study of pathology resulting from electroshock therapy of psychotic patients.   In this work neither the milliamperage nor the total time of application is comparable with that used in the present experiments.

However, there has been some work done to study the lethal effect of electrical injury under controlled conditions.   MacMahon (5) has shown that repeated sub-

lethal doses of electrical injury can cause the death of an animal. Morrison, Weeks and Cobb (6) have studied the histo-pathological effects of various types of electrical current on the nervous system. Delayed lethal effects were produced by several applications of sublethal injury over a period of a few days. The experiments of MacMahon and Morrison and his associates were done with injury limited almost entirely to the nervous system—although MacMahon does describe some changes in skeletal muscle.

While systemic toxic effects seem to have been produced in the experiments of these workers, we felt that it was desirable to place the electrodes so that the current would not transfix the entire body, but only a relatively small portion of it.

The results of our experiments favor the second postulate, that *the toxic effects observed were caused by a toxic factor circulating within the body of the test animal.* This is in accord with results of other work done in this laboratory (4) in which the dialysate of defibrinated blood from animals in heat shock was shown to have a high toxicity for rats. Extracts of injured tissues also were found to have a lethal effect on rats. Although some normal tissue extracts are toxic, it is interesting to note that the extracts from injured tissue have a higher potency than those of normal tissue. Moreover, an indication that the toxic factor of the tissues was at least similar to, and perhaps identical with, that of the dialysate of blood is found in the fact that animals injected with these materials died in a similar way.

Our own experiments with ligatures and excision of the heart corroborate the findings of numerous investigators.

That *the circulating toxic factor is a substance with thromboplastic properties* is strongly suggested by the recorded changes in the blood-clotting time following injury. It has long been recognized that disturbances in blood coagulation follow the inception of shock from trauma, burns and anaphylactic reactions. The disturbances observed and recorded, heretofore, usually have been concerned with decrease coagulability of the blood, manifested through prolongation of the clotting time (7, 8). Often the changes in blood coagulability have been referred to as being compensatory to some postulated thrombogenic tendency incited by the pathological process involved. Actually an early decrease in clotting time has been reported, at least once, in the literature, by Gahringer (9). Thus, our experiments corroborate the findings already made by others, including the important detail of the initial increase in blood coagulability. The recent work of Dragstedt and his associates (10) showed that, following burns, there was intravascular agglutination of red cells and transient and permanent thromboses of small vessels not in the burned area. More work, on traumatic injury, has shown similar results (11). These observations, we feel, favor the view that the toxic factor is a substance which has thromboplastic properties.

The foregoing statements should not be taken to mean that the end process in shock is exclusively a matter of protoplasmic clotting. Actually, no spasticity was observed in the frogs during the development of shock. What the evidence does indicate, however, is the fact that thromboplastic changes play some rôle in the development of injury shock. Although a thromboplastic toxic factor is present, the pathological process in the target tissues might not involve overt clotting alone.

The pathology might be caused by some other reaction related to the presence of the thromboplastic toxic factor, or to the compensatory 'antithrombic' factors.

SUMMARY

Profound physiological depression and death were produced in the frog by means of suitable doses of electrical injury applied either to the brain or hind legs. Evidence of a circulating toxic factor was seen in the fact that the toxic effects were prevented by interruption of the blood circulation. Disturbances in the blood-clotting mechanism following injury indicate that the toxic factor is a substance, or a group of substances, with thromboplastic properties. These results favor the concept that thromboplastic substances may be involved in the pathogenesis of shock.

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ACUTE HYPERTENSION IN DOGS WITH CEREBRAL ISCHEMIA

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CUSHING (1) demonstrated in 1903 that increased intracranial pressure can cause hypertension in dogs. Many years later Nash (2) and Volhard (3) independently showed in heart-lung-brain preparations that there is definitely a vasopressor reflex originating within the cerebral vault, and McDowall (4) demonstrated that complete cerebral anemia causes a powerful pressor response. Yet it has been the tendency by many to believe that the body's pressoreceptors are located strictly in peripheral areas (5). Experiments with sympathectomy in hypertensive patients indicate that these patients have a hyper-reactive nervous system, and there are many reasons for believing that essential hypertension is possibly neurogenic in origin (6). For this reason the present study was undertaken to elucidate the nature of the central vasopressor reflex.

METHODS

The purpose of the surgical procedures in these studies was to abolish all reflexes originating in the carotid and aortic sinuses and thereafter to study the central vasomotor reflex resulting from ischemia of the brain. This was accomplished by two methods. In the first set of animals, all of the blood supply to the brain except that through the carotid arteries was occluded. Through an incision on each side of the neck immediately above the first rib the subclavian artery was tied at its junction with the axillary artery, and all branches from the subclavian artery were independently tied and cut. The costocervical, thyrocervical, internal mammary and vertebral arteries were occluded by this procedure. The carotid sinuses were stripped without obstructing the blood flow through the internal and external carotids, and the vagi were cut at a point approximately one inch below the carotid sinuses to denervate the aortic arch. Central vasopressor reflexes were then studied by compression of the common carotid arteries. In the second series of animals the entire bifurcation of each carotid artery, including the carotid sinus and the internal carotid up to the point of its entrance into the skull, was actually removed, and the common carotids, internal carotids and external carotids were ligated. The subclavian arteries were then isolated and tied in the neck as described for the first series of animals, and all branches of the subclavian were tied except the vertebrals. There-

fore, the remaining blood supply to the brain flowed entirely through the vertebral arteries except for collateral supply through spinal and muscular channels. The cerebral reflexes were then studied by compression of these vertebral arteries. A special screw clamp was devised for this purpose so that the incisions could be closed except for the projection of a small brass tube and screwing mechanism.

Because the respiration of the animals often ceased when the brain became ischemic, it was necessary to insert an intratracheal cannula for artificial respiration. The animals were anesthetized with sodium pentobarbital to a stage of medium surgical anesthesia. The experiments progressed over a period of 4 to 7 hours. During this time, approximately one half of the animals suffered a reduction in blood pressure below normal because of the extensive operative procedures. Nevertheless, these animals had essentially the same responses found in the other animals though quantitatively smaller. To insure that the carotid sinus areas were completely denervated, they were painted after stripping with a 1 per cent solution of phenol and electrically stimulated to prove that no further neurogenic connections existed from these areas. Blood pressure was recorded from a femoral cannula.

RESULTS

After standardizing the surgical procedures, various studies of the vasopressor reflexes were made in 16 animals as follows:

A. *Acute occlusion of the common carotids with the remaining cerebral circulation intact* produced the well-known results which are generally ascribed to the carotid sinus reflex. Clamping of either one of the common carotids produced a rise in blood pressure of approximately 6 to 10 mm. Hg. Occlusion of both carotids simultaneously produced an average rise in 12 animals of 26 mm. Hg. This rise in blood pressure was maintained for the duration of carotid occlusion regardless of how long the carotids were compressed, and the blood pressure returned to normal immediately after release of the clamps.

B. *Acute occlusion of the common carotids after the vertebrals and other branches of the subclavian had been tied* produced, in 8 animals, an average rise of blood pressure of 57 mm. Hg. The character of this rise in blood pressure was slightly different from that obtained before the vertebrals and accessory circulation had been occluded. In general, the blood pressure rose rapidly for 15 seconds, then slowly for approximately one minute, after which time there was a slow fall over a period of 8 to 15 minutes back to normal or below normal. At the end of each period of occlusion there was usually a 30-second period of compensatory subnormal pressure ranging 20 to 30 mm. below normal. Cerebral ischemia in these experiments was complete except for minor blood flow through collateral channels. For this reason, even the vasopressor centers probably became functionally inactivated after a few minutes, resulting in the slowly falling pressure. The greater rise in blood pressure after the vertebrals had been ligated than before could be explained by two possible mechanisms. First, there is the possibility that back pressure in the carotid sinuses, from the circle of Willis, was less in the second case. Second, there is possibly a pressoresponsive area located in the brain in addition to those in the peripheral circulation.

C. *Acute occlusion of the common carotids with the vertebrals tied and carotid*

sinuses denervated still caused a rise of blood pressure averaging 30 mm. Hg in 7 animals. In this instance the carotid sinuses were completely inoperative and the aortic sinuses, though still intact, were actually opposing the rise in blood pressure. The character of the blood pressure response was essentially the same as that noted before the carotid sinuses were denervated, though the height was less and the rapidity of rise was slightly decreased. Likewise when the clamps on the carotids were removed, the blood pressure fell rapidly and markedly to a level 20 to 30 mm. below normal, returning thereafter to normal in approximately 15 to 30 seconds.

D. *Acute occlusion of the common carotids with the vertebrals tied, the carotid sinuses denervated and both vagi cut* caused a rise in blood pressure averaging 46 mm. of mercury in 6 animals as shown in table 1. The blood pressure, after the vagi were cut, rose much more rapidly than before, reaching a maximum in approximately 30 seconds rather than in one minute and 15 seconds. The height of the pressure was approximately one and one-half times the blood pressure rise before the vagi were cut. Cutting the vagi removed two factors in the neurogenic control of blood pressure. First, it removed the tonic and reflex effect of the vagi on the heart. This caused a variable rise in blood pressure at the time of cutting the vagi. Second, cutting the vagi denervated the aortic arch. This removed the buffering action of the pressoreceptors of the aortic arch and resulted in a higher and more rapid rise in pressure after carotid occlusion. Blood pressure readings in this category were often over 200 and, in one animal, reached a mean pressure of 260 mm.

E. *Acute occlusion of the vertebrals with the carotid sinuses totally ablated and the carotids, subclavians, costocervicals, and thyrocervicals all tied* produced an average rise in blood pressure in 4 animals of 15 mm. Hg. In this instance the carotid sinuses had been completely removed and, therefore, could have had no effect whatsoever in producing this rise in blood pressure. The character of the rise in blood pressure was the same as that previously described when the brain was almost completely ischemic except that the total rise was less than that previously discussed.

F. *Acute occlusion of the vertebrals in the 4 animals noted in paragraph E after both vagi had been cut* caused a rise in blood pressure averaging 32 mm. Hg. In this set of observations, in which both the action of the vagi on the heart and the buffering action of the aortic arch had been removed, the result was an exaggeration of the central vasopressor response.

G. *The entire neck except the spinal column was sectioned in two of the animals which already had both carotid sinuses ablated, the carotids, subclavians, costocervicals and thyrocervicals all tied, and the vagi cut.* After this procedure, the average rise in blood pressure on occlusion of the vertebrals was 76 mm. Hg as noted in table 1. It appeared that the collateral circulation increased much more rapidly when the experiment was carried out by ablating the carotid sinuses first rather than by tying the vertebrals first. This is reasonable because the small vertebrals could not adequately supply the entire head with blood. While sectioning the neck, even though the carotids had been tied and the vertebrals clamped, numerous small but profusely bleeding arteries were found throughout the muscles.

H. *Control observations.* After the buffering action of the carotid sinuses and the aortic arch had been removed, it would be possible for the increase in peripheral

·resistance upon clamping the carotids to be partly responsible for the rise in blood pressure.   Therefore, in 4 of the animals which had had the carotid and aortic sinuses denervated, one femoral artery was suddenly occluded.   In not one of these 4

TABLE 1.   EFFECT OF OCCLUDING THE CEREBRAL BLOOD SUPPLY AFTER THE CAROTID SINUSES AND THE AORTIC ARCH HAD BEEN DENERVATED

DOG NO.	VESSELS OCCLUDED	DURATION OF OC-CLUSION	B. P. BE-FORE OC-CLUSION	MAXIMUM LEVEL OF B. P. DURING PERIOD OF CERE-BRAL ISCHEMIA	REMARKS
		min.	*mm. Hg*		
5	carotids	13	140	166	Pressure had fallen to 105 at 13 min.
6	carotids	4	156	206	Pressure fell to 110 on release of occluding clamps
		14	150	226	Pressure had fallen to 70 at 14 min.
7	carotids	3	154	184	Fell to 130 on release of clamps
		10.	160	214	Pressure maintained, respiration did not cease
8	carotids	5	104	140	Rapid fall to 70 after release
9	carotids	2	166	234	Rapid fall to 130 after release
		3½	154	224	Same rapid fall
		25	184	260	Sustained blood pressure rise and sustained respiration
10	carotids	2	174	234	Rapid fall to 130 after release
		4	154	220	Same rapid fall
		22	170	200	After tying external carotids. Sustained rise in blood pressure and sustained respiration
14	vertebrals	3	100	150	Slow fall back to normal after release of clamps
		7	94	172	Slow fall back to normal after release of clamps
15	vertebrals	1½	60	168	Slow fall after release of clamps
		2	104	170	Slow fall after release of clamps

In *animals 5, 6, 7, 8, 9*, and *10*, the vertebrals, costocervical, thyrocervical and subclavian arteries had been ligated.   In *animals 14* and *15*, the carotid bifurcations had been removed and the necks had been entirely sectioned except the vertebral arteries and the spinal column.   The blood pressure levels reached their maximum height between 30 sec. and 5 min. after which they usually began to fall at a very slow rate.

animals was there a perceptible rise in blood pressure although the blood flow through the femoral was approximately equal to one half the total blood flow through both carotids and equal to considerably more than the blood flow through both vertebrals.

Tying the external carotids in 3 animals did not qualitatively change the cerebral

vasopressor response on occlusion of the carotids. Quantitatively, however, the response was slightly decreased. Because the internal carotids in dogs are extremely small arteries in comparison with the external carotids, it is reasoned that the brain probably receives a considerable proportion of its blood supply through anastomoses from the external carotids as well as through the internal carotids.

At the conclusion of one of the experiments in which the animal had had its entire neck sectioned and in which the carotid arteries as well as the vertebral arteries had been ligated, the entire spinal column was rapidly sectioned between vertebrae *C 2* and *3*. Although both vertebrals had been ligated at their origin immediately above the first rib, the animal bled to death through these vertebrals in approximately two minutes, thus showing that there was still a marked anastomotic supply between the cephalad end of the vertebrals and blood vessels of the thoracic region. This could easily have occurred because the neck had been sectioned in the region of *C 2* and *3* rather than at the point at which the vertebrals had been tied. The collateral blood supply to the brain appears to be exceptionally well developed. This makes it difficult to state the precise degree of cerebral ischemia which occurs after carotid and vertebral ligation.

I. *The effect of cerebral ischemia on respiration* varied with the duration and degree of ischemia. In approximately two thirds of the animals having the carotids, the vertebrals and accessory blood vessels occluded, respiration ceased between 45 seconds and 8 minutes after occlusion. In the other one third of the animals the respiration continued indefinitely at a slow rate. The degree of hypertension produced by cerebral ischemia was greatest in those few animals which were on the verge of total respiratory arrest but did not actually stop breathing. Likewise, the pressure response in these animals was sustained over a longer period of time than in the others. This indicates that there is a particular point in cerebral ischemia at which high levels of blood pressure can be maintained, whereas greater ischemia than this will cause a fall in blood pressure due to functional inactivation of those cells which cause the vasopressor response. During all periods of respiratory arrest, artificial respiration was instituted to prevent systemic anoxia.

J. *Prolonged cerebral ischemia in those animals which experienced complete respiratory arrest* caused, within a period of 8 to 14 minutes, a complete medullary paralysis. The blood pressure levels by this time had fallen to approximately 70 mm. Hg and clamping or releasing the arteries to the brain caused no further blood pressure responses. The animal was thereafter essentially a spinal animal and of no further use for these acute experiments.

K. *The pulse rate* in total cerebral ischemia invariably decreased after approximately 45 seconds of arterial occlusion. Before the vagi were cut, this decrease was often as much as 30 per cent. It was still present, however, even after the vagi were cut, though usually around 5 per cent in these instances rather than up to 30 per cent. Before the vagi were cut there also was often a rapid rise in blood pressure for the first 30 seconds, a small fall in blood pressure for the next 30 seconds, and then a secondary rise to higher levels at $1\frac{1}{2}$ minutes. After cutting the vagi this blood pressure dip was still noted to a slight degree in several of the animals, and it was associated with mild slowing of the pulse rate.

DISCUSSION

Long before the description of the carotid sinus reflex, the pressor response elicited by cerebral ischemia had become well known and was extensively reviewed by Winkin (7). Most of these experiments became invalidated with the discovery of the carotid sinuses, because clamping of the common carotids, which was almost always the experimental technic, caused both the cerebral ischemic response and the carotid sinus reflex response. McDowall, however, in a series of experiments designed to study the chemical control of the vasomotor center, demonstrated that a striking elevation of blood pressure could still be obtained by occluding the blood supply to the brain peripheral to the carotid sinuses and that the response persisted after the carotid sinuses had been denervated (4). It has been the purpose of the present set of experiments to evaluate this cerebral ischemic pressor response in relation to the better known peripheral pressoreceptor reflexes.

Comparison of the results of cerebral ischemia produced by arterial occlusion with those of ischemia produced by increased cerebrospinal fluid pressure in Cushing's experiments (1) is striking. There is the same slow rise in blood pressure in both procedures as well as the vagal slowing of pulse rate in the early stages. Likewise, there is the same blood pressure dip which often occurs simultaneously with the pulse slowing. The changes which take place after cutting the vagi are also the same, that is, the blood pressure rise is much more rapid, and the vagal slowing and early blood pressure dip are almost completely abolished. Cushing's original conclusion, that increased cerebrospinal fluid pressure causes the blood pressure response by producing cerebral ischemia rather than by some other mechanism, agrees quite accurately with observations in the present experiment.

Location of the centers responsible for the pressor response in the cerebral vault might be in the actual cerebral nuclei or possibly in the arterial system of the brain. It is well known that stimulation of certain areas of the hypothalamus, the mesencephalon, the pons and the medulla will cause either a rise or fall in blood pressure depending on the point of stimulation (8). It is therefore reasonable to assume that the location of the vasopressor centers concerned in the present observations could be in one of these areas. The fact that respiratory depression and rise in blood pressure correlate very closely indicates that the medulla is the area possibly concerned.

The presence of *arterial* pressoreceptor areas, similar to the carotid sinuses, inside the cerebral vault is untenable for two reasons. First, the rise in blood pressure in cerebral ischemia is usually somewhat slower than the rapid carotid sinus response, and the ischemic response often is not maintained as is the carotid sinus response. Second, characteristics of the blood pressure response in cerebral ischemia are exactly the same as those which occur when the cerebrospinal fluid pressure is increased. In this latter instance the applied pressure is external to the blood vessels, and the blood pressure should fall rather than rise if pressoreceptor areas similar to the carotid sinuses should exist within the arterial tree of the brain.

Whether the pressor response is due to actual pressoreceptor nuclei or to chemoreceptor nuclei responding to metabolic effects of hypotension is impossible to state.

The long, slow rise in blood pressure seen in the responses of figure 2 would imply a chemoreceptor system. Also, McDowall's work (4) on the chemical control of the vasomotor center indicates that carbon dioxide concentration is an important factor. On the other hand, very rapid responses occurred in a few animals as illustrated in figure 1d. These responses are more characteristic of a pressoreceptor system but not necessarily so. It is difficult to imagine the mechanical construction of a nerve
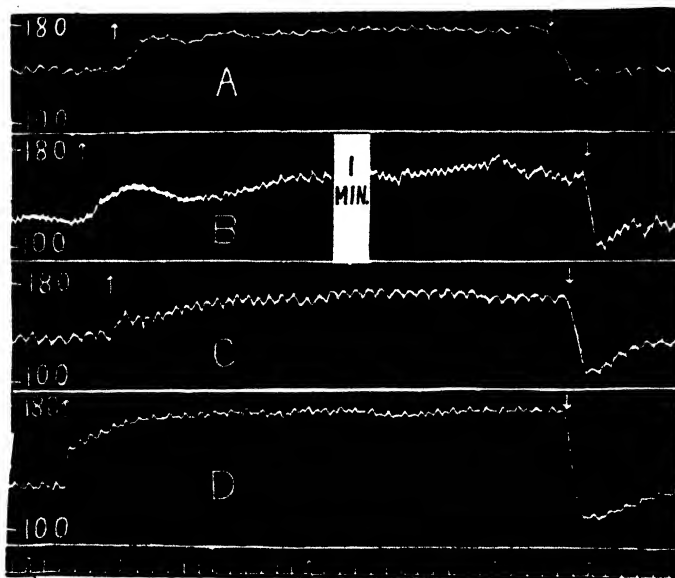
Fig. 1. BLOOD PRESSURE RESPONSES during progressive stages of the experiment: *a*) Clamping of both common carotids with the animal otherwise intact; *b*) clamping of both common carotids with the vertebrals tied; *c*) clamping of both common carotids with the vertebrals tied and the carotid sinuses denervated; *d*) clamping of both common carotids with the vertebrals tied, the carotid sinuses denervated and the vagi cut. Note in *b* the dip in blood pressure at approximately 40 seconds; this occurred frequently when the brain was almost totally deprived of blood flow but was usually entirely abolished after the vagi were cut. Note, also, the compensatory subnormal pressures after the clamps were removed from the carotids. (Time intervals—5 sec.)

cell which can respond to pressure, though such is not an impossibility. Regardless of which type of system is responsible for the pressor response, the activity of the system is rapid enough to be of protective value in animals with a falling blood pressure.

It is probable that the pressor response in cerebral ischemia is mediated through the sympathetic nervous system. The very rapid and marked fall in blood pressure when the clamps are removed from the occluded arteries indicates that the response is neurogenic rather than humoral. The vagi, of course, had been cut in these experiments and therefore could not have been concerned. Also, it has been adequately demonstrated that the rise in blood pressure due to the similar condition of increased cerebrospinal fluid pressure is mediated through the sympathetics (1, 9). Further-

more, McDowall (4) demonstrated by several methods of cerebral asphyxia that the response is opposed by removal of portions of the sympathetics.
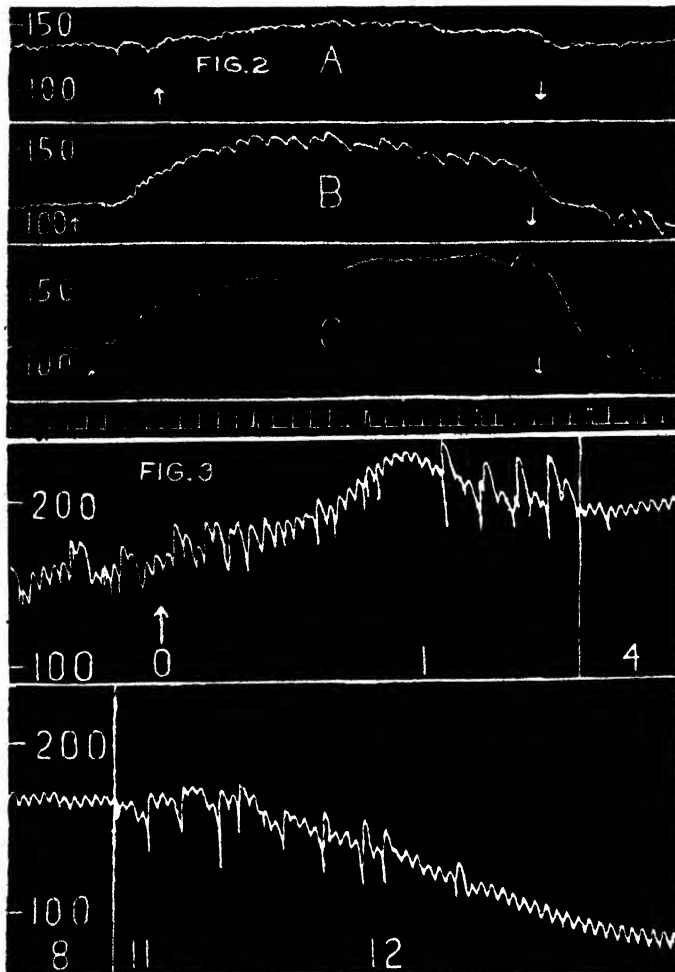
Fig. 2. BLOOD PRESSURE RESPONSES after the carotid sinuses had been totally ablated: *a*) Clamping of both vertebrals with both carotid bifurcations including the sinuses removed; *b*) clamping of both vertebrals with both carotid sinuses ablated, the vagi cut and part of the soft tissues of the neck sectioned; *c*) clamping of both vertebrals with both carotid sinuses ablated, the vagi cut and the neck, except the spinal column, completely sectioned. Note the change in response as the collateral circulation was destroyed and the aortic sinus denervated. (Time intervals—5 sec.)

Fig. 3. BLOOD PRESSURE EFFECT of prolonged cerebral ischemia. Medullary paralysis with resultant spinal levels of blood pressure occurred in 8 to 14 minutes in all animals if the ischemia was sufficient to cause respiratory arrest.

The present experiments indicate forcefully that the peripheral pressoreceptors are not the only major neurogenic pressoresponsive system. Indeed, it is even questionable whether the carotid and aortic sinuses are as powerful as the cerebral centers.

It is regrettable that many of the experiments to produce chronic hypertension by carotid sinus denervation have been carried out by actual ablation of the carotid bifurcation (10–12). This procedure could easily cause a low-grade deficiency in blood supply to the brain, and what the effects of this may be have not been determined. For this reason, and because the results of chronic experiments with sinus denervation have been extremely variable (10–14), the precise function of the carotid sinuses, the aortic arch and the mesenteric pressoreceptors (15, 16) is still quite beclouded.

The relation of these experiments to essential hypertension in man is of interest. Certainly, no one has succeeded in implicating the carotid sinus mechanism as a causative factor. Yet, excessive neurogenic pressor responses in such patients have been demonstrated by postural and ice water tests (6). Also, it has been shown many times that spinal (17), caudal (18), and differential block (19) anesthesia, which effectively block the sympathetic nervous system, will cause a marked blood pressure fall in many hypertensive patients, whereas these procedures hardly affect the blood pressure of normal individuals. These observations indicate a cerebral origin of excess sympathetic activity in hypertensive patients. Because stimulation of an extremely small area of the medulla has been shown to cause extreme changes in blood pressure (8), there is no reason to doubt that local vasospasm or arteriosclerotic occlusion of a small blood vessel might be responsible for the hyperactive sympathetics. Indeed there might well be a vicious cycle with localized vasospasm initiating a neurogenic hypertension and this in turn reflexly increasing the vasospasm.

Recent war experiments concerning the effect of increased gravitational force on the body indicate that man's ability to rapidly adjust the blood pressure under changing forces is more highly developed than that of lower animals (20). This is reasonable because of man's erect posture, and it might also explain why essential hypertension is principally a disease of mankind.

Chronic hypertension has been produced in dogs by progressively occluding the arterial blood supply to the brain (21). To cause this state, it has been necessary to occlude even the anterior spinal artery, and it might be reasoned that such extreme ischemia could hardly exist under natural conditions. One must remember, however, that very minute areas of the brain may exert powerful pressor effects, and severe localized ischemia in these areas can easily occur.

SUMMARY

A marked rise in blood pressure occurs in response to acute cerebral ischemia. This response is still present after reflexes from the carotid sinuses have been abolished. Quantitative data indicate the cerebral pressoreceptor response to be as powerful as the carotid sinus response though of a slightly different character. Respiration is also depressed by acute cerebral ischemia, and the rise in blood pressure generally is greatest when the respiration is barely present. Prolonged cerebral ischemia produces complete medullary paralysis causing the blood pressure to fall to levels of a spinal animal. It is postulated that the centers responsible for the blood pressure response are located in the medulla. The blood pressure response due to cerebral ischemia is almost identical with that shown by Cushing to occur

in increased cerebrospinal fluid pressure. The possible relationship of these observations to essential hypertension is discussed.

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ELECTRICAL STIMULATION OF THE NEURAL MECHANISM REGULATING SPASMODIC RESPIRATORY ACTS IN THE CAT[1],[2]

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IN 1939 Wang and Ranson (1) mapped the reactive points for certain autonomic responses to stimulation of the cat's brain stem and reported as an incidental observation an unusual augmented respiratory response elicited at the level of the calamus scriptorius. Since then Pitts, Magoun, and Ranson (2), Brookhart (3) and others (4, 5) have carefully explored the medulla for various respiratory effects but have made no mention of this particular type of response.

During the past year, the medulla oblongata of the cat has been stimulated in this laboratory with the aid of the Horsley-Clarke stereotaxic instrument, and a spasmodic respiratory response similar to that reported previously by Wang and Ranson (1) has been obtained. This recent work indicates that the response may be further characterized as a rhythmic explosive expiratory act as typically seen in coughing, sneezing and retching. It has been repeatedly and consistently elicited on stimulation of definite loci in the dorsolateral portion of the medulla. The purpose of this work is to localize the neural structures involved, to study in detail the physiological nature of the response, and to determine the conditions favoring its occurrence.

METHODS

Of the 40 experiments performed in this study, 25 were done on cats anesthetized with nembutal (20 mgm. per kgm.) given intraperitoneally. This was usually supplemented with ether during the operative procedure. The remaining 15 experiments were carried out on decerebrate cats prepared by midcollicular transection (6) under ether anesthesia. The cerebellum was exposed widely by removing with trephine and rongeurs the portion of the occipital bone from the foramen magnum to the lambdoidal ridge. The interior of the medulla was stimulated with a bipolar enamelled wire electrode which was oriented by means of the Horsley-Clarke stereotaxic instrument (7). The electrode was mounted in a rotatory electrode carrier (8) and was inserted through the cerebellum into the medulla at a forward inclination of 15 degrees. The stimulating current was supplied by a thyratron stimulator (9) which was calibrated with a cathode ray oscilloscope. Since, as will be shown, it was necessary to leave the glottis undisturbed, a pneumograph type of recording offered certain advantages over the use of a spirometer. Taking into consideration the misleading effects which extraneous body movements frequently produce in ordinary pneumograph tracings, as was pointed out by Pitts *et al.* (2), a system was devised in which blood pressure cuffs (8 cm.) were adapted as thoracic and abdominal pneumographs; these were used in conjunction with gravity writing tambours. That this method gave reliable recordings is indicated in figure 1, in which simultaneous pneumograph and spirometer records made under a variety of conditions are shown. At the termi-

nation of each experiment the cat's brain was perfused first with saline, then with formalin to fix the tissue in situ. This technique reduced to a minimum any distortion of the brain which might occur on subsequent handling. The points reactive to stimulation in each medulla were localized by identifying the electrode punctures in Weil-stained serial sections cut in the plane of the punctures.

RESULTS

In addition to the maintained inspiratory and expiratory responses localized by Pitts and his co-workers, faradic stimulation of the lower brain stem has yielded a characteristic spasmodic respiratory response which is specific for the dorsolateral region of the myelencephalon.

Spasmodic respiratory response. To appreciate the actual nature of the spasmodic response it was necessary to avoid the use of a tracheal cannula so that the glottis could function normally. With the glottis intact, the response may be readily distinguished as a cough, sneeze or retch. Typical examples of the recorded spasmodic respiratory response are presented in figures 2 and 3. The response occurs only during the stimulation period although it may terminate before the end of stimulation. Those responses which appeared after the stimulation time were not considered typical and were not included in the localization of the reactive points. The spasmodic response consists of strong inspiratory and expiratory efforts usually occurring at a rate of one per second, but in some instances taking place at rates even as slow as one every four seconds. While the glottis is a useful indicator of the specific character of the spasmodic respiratory act, bypassing it with a tracheal cannula does not alter the recorded response to any appreciable extent. Similar responses to those elicited on direct stimulation of the medulla may be obtained by stimulating certain afferent nerves. In figure 4 the responses resulting from stimulation of the central ends of the glossopharyngeal and superior laryngeal nerves are presented for comparison with those obtained by medullary stimulation.

Localization. The results of histological identification of the reactive points from which the spasmodic respiratory response was obtained are compiled in the drawings of figure 5. These represent sections of the medulla spaced at approximately 1.5 mm. intervals extending from the level of the 6th cranial nerve caudad to the level of the 12th cranial nerve. In order to make the localization as exact as possible, only those points which gave maximal responses are plotted. It is seen that the responsive areas correspond to the descending vestibular tract and nucleus, the tractus solitarius and its nucleus, and the entering glossopharyngeal and vagal rootlets. Only sporadic or questionable responses were obtained either rostral or caudal, to the levels indicated in figure 5. The levels at which the reactive points are most concentrated are B, C, and D, corresponding to the outflows of the 9th and 10th cranial nerves. Although the same total number of points were not stimulated at each level, the data of figure 5 give a rough idea of the relative reactive density within the responsive region localized. It is not possible with the present information to subdivide the responsive region into distinct parts each separately responsible for coughing, sneezing and retching. Changes in position of the electrode in the immediate vicinity of the localized region gave no predictable pattern of responses. Thus, there were times when on moving the electrode dorsad through the medulla, the spasmodic respiratory response would appear following a typical apneusis, and on

Fig. 1. COMPARISON OF SPIROMETER and pneumograph tracings. *Top tracing:* spirometer, inspiration downstroke; *center* and *bottom tracings:* thoracic and abdominal pneumographs respectively, inspiration upstroke. A. Maintained inspiratory response; B. expiratory apnea; C spasmodic response; D. atypical delayed spasmodic response. Time signal: 15 seconds.
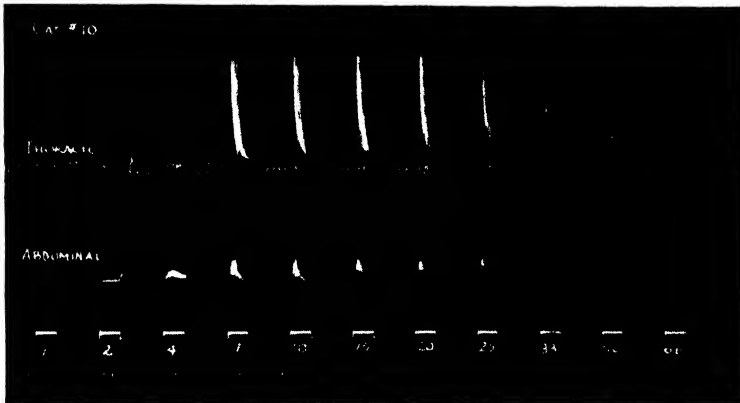
Fig. 2. EFFECT OF frequency variation of the stimulating current on the spasmodic respiratory response. Frequency, in impulses per second, indicated by the numbers under the stimulation marker. Time signal: 15 seconds. *Upper record: cat 10*, voltage is constant at 12 volts. Optimal range of frequencies is from 7 to 25 impulses per second. Note at high frequencies the inspiratory cramp in the thoracic tracing and the expiratory apnea in the abdominal tracing. *Lower record: cat 40*, voltage is constant at 8 volts. Optimal range of frequencies is from 2 to 10 impulses per second.

other occasions it might occur immediately superficial to a point yielding an expiratory apnea. In any event, the spasmodic response was always elicited most dorsally.

Frequency and voltage characteristics. The spasmodic respiratory response can be elicited solely within well defined and relatively small limits of frequency and voltage variation of the stimulating current. It can be seen from figure 2, *cat 10*,
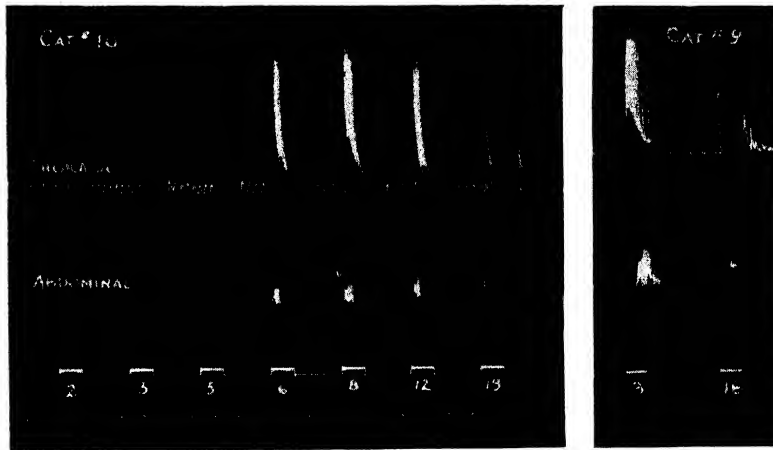
Fig. 3. Effect of voltage variation of the stimulating current on the spasmodic respiratory response. Frequency is constant at 15 impulses per second in both records. Voltage is indicated in volts by the numbers under the stimulation marker. Time signal: 15 seconds.

Fig. 4. Comparison of spasmodic respiratory responses resulting from direct medullary stimulation and the stimulation of afferent nerves. *Upper tracings:* thoracic pneumograph; *lower tracings:* abdominal pneumograph. Time signal: 15 seconds. *Cat 4,* A. Medullary stimulation at 25 volts and 10 impulses per second; B. glossopharyngeal nerve stimulation at 25 volts and 10 impulses per second. *Cat 39,* A. Medullary stimulation at 8 volts and 4 impulses per second; B. superior laryngeal nerve stimulation at 18 volts and 4 impulses per second.

that with a constant current strength of 12 volts, the response is optimal in the frequency range of 7 to 25 impulses per second. At the low frequency end, the spasmodic response appears suddenly, whereas at frequencies above 25 impulses per second it is restrained and replaced by an inspiratory cramp. Variation in voltage gives results which are similar to the effect of frequency change (fig. 3, *cat 10*). With constant frequency at 15 impulses per second the response appears suddenly at 6 volts

and at 19 volts is merged into an inspiratory cramp. It should be pointed out that the results cited are for a single experiment and that there are differences in the optimal range of frequency and voltage from animal to animal (fig. 2, *cat 40* and fig. 3, *cat 9*). Nevertheless, it must be emphasized that the range of reactive frequencies is consistently below 40 impulses per second and in any given animal lies within fairly restricted limits. The effects of voltage variation are not as specific as those produced by frequency change, yet it is significant that the response is elicitable at moderate current strengths. Anesthesia is an important factor to be controlled, for the depth of anesthesia has a strong modifying effect on the current characteristics and may possibly cause complete disappearance of the response.
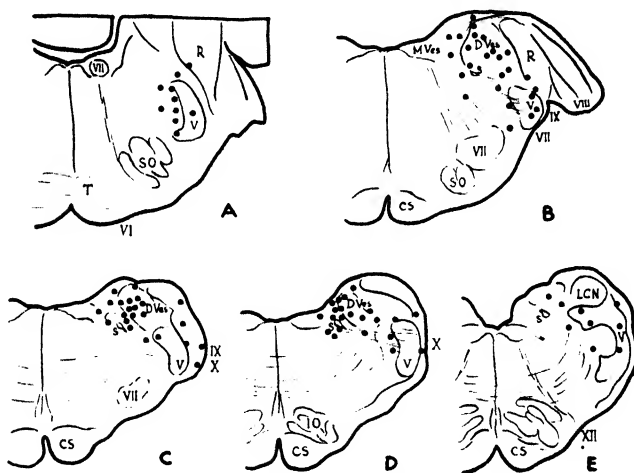
Fig. 5. SECTIONS OF THE MEDULLA of the cat spaced at approximately 1.5 mm. intervals between the levels of cranial nerves VI and XII. *Solid circles:* maximal spasmodic respiratory responses. Ratio of positive to negative experiments at each level: *A*, (4/7); *B*, (11/0); *C*, (6/2); *D*, (8/5); *E*, (2/3). *DVes*, descending vestibular tract and nucleus; *MVes*, medial vestibular nucleus; *R*, restiform body; *s*, tractus solitarius and nucleus; *SO*, superior olivary nucleus; *IO*, inferior olivary nucleus; *T*, trapezoid body; *CS*, corticospinal tract; *LCN*, lateral cuneate nucleus.

For routine exploration, a stimulating current at a frequency of 15 impulses per second and a strength of 8 volts was employed. In general, high frequency or voltage stimulation causes the spasmodic response to be converted into a characteristic inspiratory cramp, and low frequencies and voltage tend to change it into an expiratory apnea. However, numerous exceptions to this generalization have been seen. A possible effect of voltage or frequency variation which has been considered but never observed is the conversion of one type of spasmodic respiratory response to some other type, for example, coughing to sneezing.

DISCUSSION

Lumsden in 1923 (10) suggested the existence of a medullary center for active expiratory efforts similar to coughing and sneezing. His basis for this assumption is that these sudden forced respiratory acts are obtainable on stimulation of afferent fibers in the 5th and 10th cranial nerves, and that the neural structures above the

level of the striae acousticae are not essential for these acts.   In more recent years, the brain stem has been explored extensively for respiratory reactivity by direct electrical stimulation (2–5) with the sole criterion being respiratory movement for the purpose of pulmonary ventilation.   That the respiratory apparatus is also normally utilized for other important functions, as for expulsive purposes, is obvious.   These later investigators have in general used stimulating currents with frequencies too high to elicit the spasmodic respiratory response observed in this study.   Moreover, if the trachea is cannulated for spirometer recording, thus bypassing the glottis, it is quite difficult to recognize the nature of the spasmodic respiratory response and practically impossible to distinguish between the various types of spasmodic acts.

Although pneumograph recording gives only qualitative information it has the advantage of not interfering with respiratory function, and also provides a means of separating the thoracic and abdominal components of respiration.   The rôle of the abdominal musculature is of particular importance in expulsive respiratory acts. Contrary to the claim of Pitts (2) that the pneumograph cannot be used with accuracy, this recording method has been shown to faithfully reproduce a variety of respiratory activities (fig. 1).

Using appropriate stimulus frequencies and strengths, the spasmodic respiratory center has been localized within relatively sharp boundaries in the periventricular substance of the lower brain stem.   It is apparent from the illustrations in figure 5 that the distribution of the reactive points is sparser in the most caudal and rostral drawings than in the levels *B, C* and *D*.   This indicates that levels *A* and *E* represent the outlying limits of the region which has been defined by electrical stimulation as regulating spasmodic respiratory responses.   Those structures implicated in the localization correspond mainly to the descending vestibular root and its nucleus, the tractus solitarius and its nucleus and related vagal cells.   It has been shown by Pitts (11) that the method of localization used in this study is accurate to within half a millimeter.   This means that while the method has inherent limitations, it is sufficiently exact to permit the designation of specific structures with reasonable certainty. A comparison of the localization of the spasmodic respiratory center with Pitts' respiratory center shows a striking lack of conflict between the anatomical positions of the two centers.   There appears to be very little overlap at those levels in which they are coexistent.

Undoubtedly some of the responses elicited from the reactive points are the result of the stimulation of afferent fibers and tracts.   Lumsden has induced expulsive responses by electrical stimulation of the 5th and 10th nerves or by blowing air into the nostril or larynx.   Teitelbaum and Ries (12), who stimulated the glossopharyngeal nerve, described the response as an immediate marked respiratory augmentation.   The recordings presented in figure 4 leave no question as to the close similarity between the responses obtained reflexly and those resulting from direct stimulation of the reactive region in the medulla.   Nevertheless, the fact that the responsive points are concentrated within a well defined region, and that the response is still centrally elicitable after midbrain decerebration, is evidence for the existence of a spasmodic respiratory *center* in the rhombencephelon.   This view is further strengthened by an experiment performed by Lumsden in which the forced expiratory

response was produced reflexly after decerebration, demonstrating that the central coordinating mechanism must still be intact. Furthermore, direct stimulation of the pontile region gave responses which did not satisfy the criteria for spasmodic respiratory acts, thereby serving to limit the rostral extent of the spasmodic respiratory center to the caudal edge of the pons. Having thus restricted by the process of elimination the central mechanism for spasmodic acts to the myelencephelon, one must conclude that the spasmodic respiratory center lies within the reactive region shown in figure 5.

It should be emphasized that the spasmodic response can be elicited only within very specific frequency and voltage limits of the stimulating current. Although low frequency stimulation is a necessary condition for the production of the spasmodic response, the latter is not an invariable consequence nor is it an artifact associated with such stimulation. Structures outside the spasmodic respiratory center when stimulated with a low frequency current yielded no similar results. Indeed, Pitts has previously pointed out that reducing the frequency or voltage of stimulation in the medial reticular formation does not vary the form of the maintained respiratory response but merely decreases its amplitude. This is in contradistinction to the marked altering effect which variation of frequency or voltage has on the spasmodic response. It is evident from the foregoing that the frequency and voltage characteristics necessary for eliciting the spasmodic respiratory response give it a firm basis as a physiological entity. In other words, the spasmodic response represents a real and coordinated action of the respiratory musculature specifically obtainable with a specialized stimulus from well defined medullary structures.

There can be no doubt as to the close synaptic relationship of the spasmodic respiratory center with the medullary center localized by Pitts and his associates (2). Evidently there is a neuronal mechanism within the medulla which rhythmically activates the inspiratory and expiratory cells in the production of forced spasmodic acts. At the present time the importance of this mechanism in the regulation of normal rhythmic breathing can only be conjectured. Lumsden (13) postulated a pneumotaxic center in the pons. Later investigators (14–16) have further established the importance of the pneumotaxic center as a source of periodic inhibition of inspiration; they claimed that in animals with this pontile apparatus extirpated, blocking of the vagi results in apneusis. Although it is is generally agreed that the pneumotaxic center is located in the upper few millimeters of the tegmentum of the pons, direct stimulation of the pontile structures have given no obvious indications of the presence of this center. It is important to mention that the evidence for the existence of a pontile pneumotaxic mechanism has been based entirely upon complete or partial transection experiments, and that the accuracy of such procedures, in delimiting neuronal boundaries, is always questionable. Indeed, a number of workers (16, 17) have reported a modified type of rhythmic breathing in animals after pontile ablation and bilateral vagotomy. This is explained by Pitts (18) as attributable to remnants of the pneumotaxic center extending downward into the medulla. The topography of the spasmodic respiratory center in the myelencephelon, and the repetitive character of the response suggest that it may be related to the mechanism of pneumotaxis and may be a factor responsible for the residual breathing in the vagotomized and pontile transected animals.

SUMMARY

The brain stem of the cat was stimulated with the aid of the Horsley-Clarke stereotaxic instrument and a spasmodic respiratory response which is recognized as coughing, sneezing and retching has been elicited (figs. 1–4). The central mechanism regulating this response has been localized in the dorsolateral region of the myelencephelon including the structures corresponding to the descending vestibular tract and nucleus, the tractus solitarius and nucleus, and the vagal and glossopharyngeal rootlets (fig. 5). Stimulation with low frequency (less than 40 impulses per second) and moderate voltage currents favor the occurrence of the spasmodic response (figs. 2 and 3).

A possible relationship of the spasmodic respiratory center to the mechanism of pneumotaxis is discussed.

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ACTION POTENTIALS IN RAT MUSCLE WITH TWITCH TENSION POTENTIATED BY KCl TREATMENT, ADRENALECTOMY, TETANUS AND TREPPE[1]

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IN A previous study (1) it was found that the response of rat muscle to single stimuli showed a greater developed tension and a longer contraction time in adrenalectomized animals than in normal controls. The increased contraction time and the prolonged action potentials observed after adrenalectomy led to the suggestion that repetition might account for a part of the increased tension. In the same report it was shown that the response of muscle potentiated by intraperitoneal injection of KCl had a longer contraction time than the response of untreated muscle.

Brown (2) studied the effect of K on the action potential in muscle of the cat after intra-arterial injection of KCl. This investigator observed a decrease in height of the action potential but he did not report a change in duration of the action potential or an increase of contraction time of the KCl-treated muscle.

In a recent report (3) it was shown that the increased tension in responses of muscle to single stimuli in rats treated with desoxycorticosterone acetate is accompanied by repetitive action potentials. However, these potentiated responses differ from those seen after KCl treatment, adrenalectomy and tetanus in that they disappear after the third to sixth response to single stimuli.

The present study was undertaken to determine the nature of the action potentials which accompany the responses to single stimuli in muscle showing increased twitch tension as a result of KCl treatment, adrenalectomy, tetanus or treppe. A preliminary report on this study has been made (4).

METHODS

The records were obtained under ether anesthesia from immature male rats weighing 150 to 200 gm. Action potentials were recorded from the gastrocnemius muscle with a cathode ray oscillograph. Mechanical records of muscle contraction were made simultaneously using an isometric lever and optical recording as previously described (1). The cut sciatic nerve was stimulated with brief shocks three to four times threshold strength. Normal rats were injected intraperitoneally with 40 to 80 mg. of KCl per 100 gm. of body weight. The records were obtained from adrenalectomized rats after the appearance of signs of severe adrenal cortex insufficiency, i.e., weight loss and muscular weakness. A few adrenalectomized animals were allowed to drink one per cent KCl solution instead of tap water. Tetanus was produced by repetitive shocks at the rate of 200 per second for one to 10 seconds. Treppe was brought about by single shocks at the rate of one per second. As a basis of comparison, control records were made from rested muscle of normal rats.

To obtain blood for analyses, the heart was exposed and artificial respiration was given. The

blood was drawn from the left ventricle into a syringe moistened with heparin. The plasma was separated immediately by centrifugation.

Plasma and muscle K were determined by the potassium phosphotungstate method of Van Slyke and Rieben (5). This method was modified for determination of muscle K. The muscle was placed in a platinum crucible and dried to constant weight in an electric oven at 105°C. About 0.5 cc. of 4 N H_2SO_4 per gm. of muscle was added and the crucible was placed under an Infra Rediator for about an hour. The crucible was then transferred to a cold muffle furnace and the muscle was ashed at 480 to 500°C. The ash was dissolved in 1 to 2 cc. of water containing one drop of concentrated HCl. The solution was transferred to a 10 cc. volumetric flask containing a drop of phenolphthalein indicator and was made up to volume. $Ca(OH)_2$ was added until the solution became slightly alkaline. The solution was then left over night and was filtered the next day. Further dilutions were made depending on the size of the muscle. For 1- to 2-gm. muscles a one to 100 dilution was used. Aliquots of 3 cc. were taken for analyses. For determination of muscle Na, 3 cc. aliquots of the ashed muscle were determined gravimetrically as the uranyl zinc acetate by the Butler and Tuthill (6) method. Plasma Cl was determined by the Sendroy (7) method. The powdered muscle for Cl determination was mixed with five times its weight of sodium carbonate and ashed at 450°C. The ash was dissolved with warm water made slightly acid with nitric acid and Cl was determined with the Volhard titration.

The extracellular water was calculated from the ratio of muscle Cl to the concentration of Cl in the plasma water. It was assumed that all muscle Cl is extracellular. The water content of the plasma was considered 94 per cent. The calculations used to obtain the data shown in table 2 were essentially the same as those described by Hastings and Eichelberger (8) and by Lowry and Hastings (9).

RESULTS

KCl treatment. In the first series of experiments action potential records were obtained with both recording electrodes placed in the belly of the gastrocnemius. The leads in this position have been found more effective than the belly and tendon leads for detection of repetitive discharges involving only a small portion of the muscle fibers (3). Results typical of the findings in 5 rats are shown in figure 1A in the record taken 40 minutes after the intraperitoneal injection of 80 mg. of KCl per 100 gm. of body weight. Although the mechanical response which accompanied this record showed a 60 per cent increase of twitch tension no repetitive discharges were seen.

The decrease in height and increase in duration of muscle action potential produced by prolonged treatment with KCl are marked when one lead is placed in the belly of the muscle and the other lead is placed in the tendon or in a distant point (fig. 1B). The gradual decrease in height and increase in duration of action potential are shown in the records in figure 1C. These changes in electrical response were accompanied by progressive increases of contraction time and twitch tension. The percentage increase of twitch tension was approximately equal to the percentage increase of contraction time in the KCl-treated muscle. For example, the mechanical response recorded 55 minutes after KCl injection (fig. 1C) showed about 65 per cent increase in contraction time and about 70 per cent increase in twitch tension.

When belly and tendon or belly and distant leads were employed, action potential records were rather smooth before KCl treatment if the belly lead were placed near the surface of the muscle (fig. 1B). Multiple peaks frequently appeared in the electrical records, however, when the mechanical responses of the muscle were potentiated by K (fig. 1B). By placing the belly lead in the interior of the muscle it was

possible to obtain action potential records with multiple peaks from the untreated muscle. These peaks, or components, showed temporal dispersion after KCl treatment (fig. 2, A and B). The dispersion was not uniform, however, because the peaks tended to overlap and partially lose their identity. The latent period between the stimulus and the beginning of the action potential was two to three times longer after prolonged KCl treatment (fig. 2A).

The relationship between decreases of action potential height and increases of action potential duration was not linear. The height of the action potentials diminished *more* rapidly during the first 20 minutes after KCl injection and less rapidly as potentiation of muscle tension progressed. On the other hand, the duration of the action potentials increased *less* rapidly during the first 20 minutes and more rapidly during the subsequent period. These changes are shown in A and B of figure 3
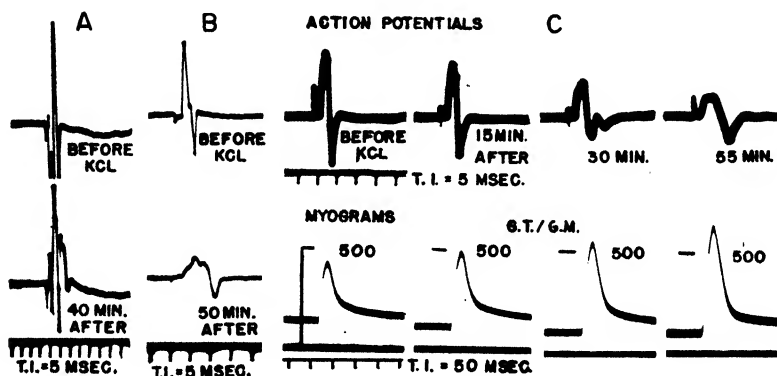
Fig. 1. EFFECTS OF INTRAPERITONEAL INJECTION of 80 mg. of KCl per 100 gm. of body weight on the muscle action potential and twitch tension of the gastrocnemius of the rat. The muscle was stimulated with single indirect shocks 3 to 4 times threshold strength. *A.* Action potentials with both leads in the belly of the muscle. *B.* Action potentials with one lead in the belly of the muscle and the other lead in a distant point of the animal. *C.* Action potentials (*above*) with belly and tendon leads and corresponding mechanical records of twitch tension (*below*).

which is a diagram of the average and range of responses induced in 3 different animals. It is interesting to note that the marked diminution in height of action potential during the first 20 minutes of KCl treatment is similar to that obtained by Brown (2) in the cat when intra-arterial injections of KCl were made.

Marked increases of plasma K were found 15 minutes after KCl injection (table 1). Slight increases of muscle K (table 1) and intracellular concentration of K (table 2) were induced by 15 minutes of KCl treatment. Application of the data given in table 2 shows that consideration of changes in cell weight may account for 70 per cent ($118 \times 900/874 = 121.5$) of the increase of intracellular K. On the basis of changes in intracellular water 75 per cent ($160 \times 664/639 = 166$) of the increase of intracellular concentration of K may be due to dehydration of the cells. The level of plasma K found in animals sacrificed about one hour after injection of KCl was slightly higher than that seen 15 minutes after KCl injection. Moreover, a greater increase of intracellular K was observed in the muscle of animals treated for one hour with KCl. The data in table 2 indicate that about 50 per cent of the in-

crease in intracellular K concentration, in muscle treated for one hour, is due to movement of water out of the cells.   The percentage increase of extracellular K concentration greatly exceeded the percentage increase of intracellular K concentration in all muscle treated with KCl (table 2).   Plasma and muscle Cl increased and plasma and muscle Na decreased during KCl treatment.   Although the total water content of muscle was unchanged (table 1), the intracellular water was decreased and the extracellular fluid was increased by KCl treatment.
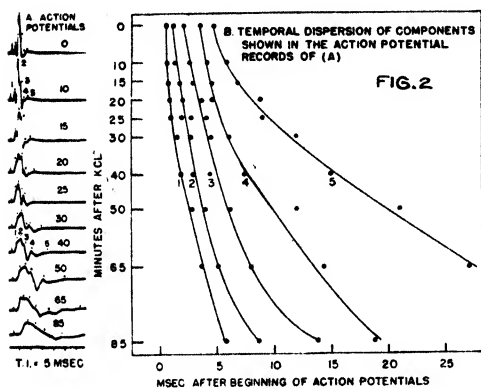
Fig. 2. EFFECTS OF INTRAPERITONEAL INJECTION of 80 mg. of KCl per 100 gm. of body weight on the gastrocnemius muscle action potential with one lead electrode placed in the center (*interior*) of the belly in such a position that several discreet points (1–5) were seen on the records.   The other lead was in the tendon.   The muscle was stimulated with single indirect shocks 3 to 4 times threshold strength.   *A*. Action potentials with the time of recording indicated as minutes after KCl injection.   *B*. Graph showing the temporal dispersion of the components in the action potentials shown in *A*.   (The twitch contraction time increased from 18 to 32 msec. and the twitch tension increased 80 per cent during the progress of the experiment.)

FIG. 3. DIAGRAMS SHOWING THE RELATION OF TWITCH TENSION to action potential height (*A*) and action potential duration (*B*) in 3 rats injected with 80 mg. of KCl per 100 gm. of body weight. The circles show averages and the horizontal bars show the ranges of values.
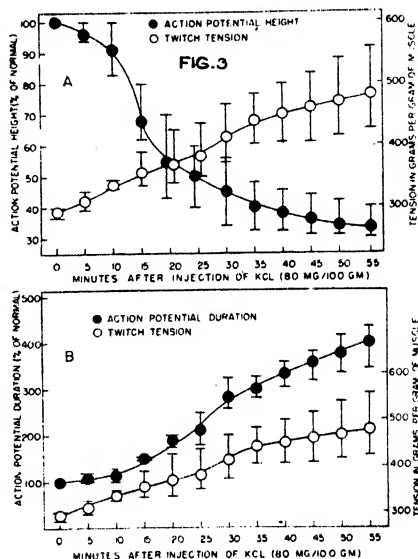
Effects of adrenalectomy.   It has been found that the muscles of adrenalectomized rats develop tension 40 to 50 per cent greater than that seen in normal muscle in response to single stimuli (1).   The muscle action potentials obtained with belly to tendon leads in the adrenalectomized animals show an increased duration when compared with the action potentials from similarly placed leads in normal muscle.   These experiments were repeated in 5 animals showing severe adrenal insufficiency and the previous results were confirmed (fig. 4, A and B).   Although the height of the action potentials in both adrenalectomized and normal animals varied considerably, presumably depending upon the position of the lead electrodes with respect to the adjoining muscle fibers, the duration of the action potentials was less variable and it was

consistently greater in the adrenalectomized than in the normal animals.   In an attempt to exaggerate the increase of potassium in adrenalectomized animals 1 per cent of KCl was given in the drinking water.   The plasma and intracellular K levels and the duration of the muscle action potentials (figure 4C) were increased by the

TABLE 1. AVERAGE VALUES FOR ANALYSES OF RAT MUSCLE AND PLASMA

NO. OF RATS	MUSCLE (PER KG. OF FRESH TISSUE)				PLASMA (PER LITER)		
	water	K	Na	Cl	K	Na	Cl
KCl treatment[1] for 15 minutes							
	gm.	mM	mM	mM	mM	mM	mM
3	765 ±0.9[2]	109.3 ±0.4	16.3 ±0.6	15.7 ±0.7	13.4 ±0.7	144 ±0.7	113 ±3.2
KCl treatment for 55 to 65 minutes							
3	764 ±1.0	112.7 ±1.8	14.1 ±0.9	16.0 ±1.1	14.8 ±0.6	142 ±1.2	118 ±0.4
Control rats							
4	763 ±1.1	106.5 ±0.8	17.2 ±0.6	12.0 ±0.5	4.5 ±0.2	150 ±2.3	110 ±1.3

[1] 80 mg. of KCl (2%) per 100 gm. of rat were injected intraperitoneally.

[2] Standard error of the mean = $\sqrt{\dfrac{\Sigma d^2}{n(n-1)}}$.

TABLE 2. AVERAGE VALUES FOR DISTRIBUTION OF WATER AND POTASSIUM IN MUSCLE.   DATA DERIVED FROM TABLE 1

NO. OF RATS	EXTRACELLU-LAR FLUID	TOTAL CELL WT.[1]	INTRACELLULAR WATER		K	K	K
	Per kg. muscle	Per kg. muscle	Per kg. muscle	Per kg. of cells	Per kg. extracellular water	Per kg. of cells	Per kg. intracellular water
KCl treatment for 15 minutes							
3	126 ±3.3	874 ±3.3	639 ±4.2	732 ±1.9	13.5 ±0.77	123 ±0.6	168 ±0.9
KCl treatment for 55 to 65 minutes							
3	130 ±2.9	870 ±2.9	635 ±4.4	730 ±2.1	14.9 ±0.65	128 ±2.9	174 ±3.1
Control rats							
4	100 ±4.1	900 ±3.2	664 ±3.8	737 ±0.9	4.52 ±0.34	118 ±1.4	160 ±2.0

[1] The values in this column include all extracellular material (i.e., collagen and elastin) not calculated with extracellular fluid.

drinking of KCl solution.   However, the developed tension was about equal to that observed in adrenalectomized animals receiving tap water.   A study in 5 adrenalectomized animals with both leads placed in the belly of the muscle showed single muscle action potentials in response to single shocks.

Effects of tetanus.   Post-tetanic responses of normal muscle stimulated with

200 shocks per second for two seconds or less were accompanied by action potentials with reduced height and unchanged duration. The responses after tetanic stimulation for three seconds showed action potentials with further decrease in height and with slight increase of duration (fig. 5A). Further increases of the period of tetanic stimulation gradually decreased the height and increased the duration of post-tetanic action potentials. A marked increase of duration was obtained by 10 seconds of tetanic stimulation (fig. 5B). The time required for return to action potentials similar to the pretetanic ones did not appear to be related to the duration of tetanus.

Fig. 4. EFFECTS OF ADRENALECTOMY and of adrenalectomy plus a one per cent KCl drinking solution on the action potentials and twitch tension. *A*. Comparison of action potentials and twitch tension in the gastrocnemius muscle of normal (N) and adrenalectomized (A) rats. *B*. Comparison of action potentials in the gastrocnemius muscle of normal (N) and adrenalectomized-KCl-treated (A + 1 % KCl) rats. The action potential records were made with belly and tendon leads and they are superimposed.

Fig. 5. ACTION POTENTIALS AND MYOGRAMS showing the effects of tetanus on post-tetanic responses. *A*. Responses before and after 3 seconds of tetanus at 200 shocks per sec. *B*. Responses before and after 10 seconds of tetanus at 200 shocks per second. Stimulation was indirect.
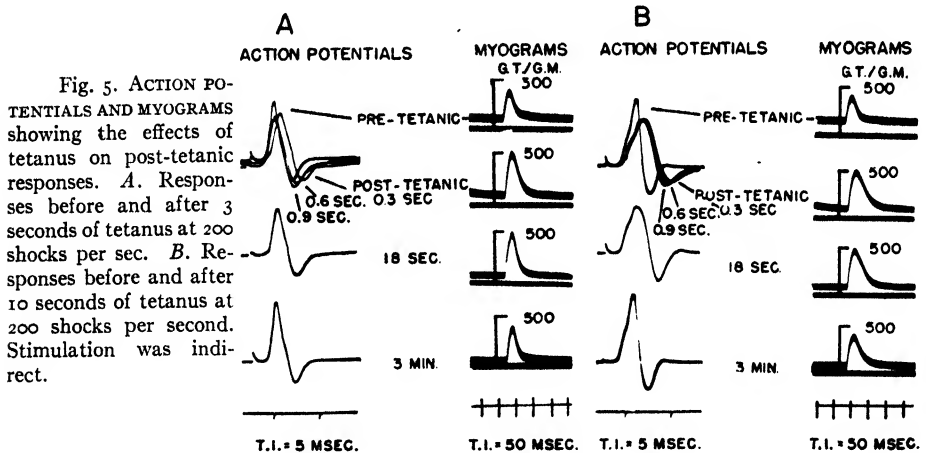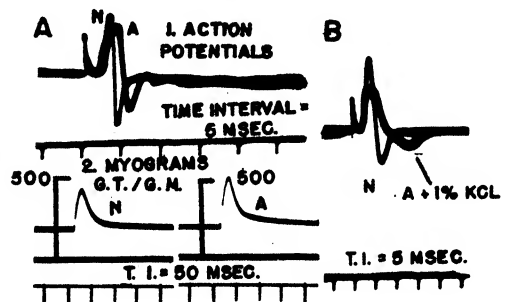
For example, the time for recovery of the pretetanic form of the action potential was approximately 3 minutes both for a 3-second tetanus and for a 10-second tetanus (fig. 5, A and B). The marked increases of action potential duration observed after prolonged tetanic stimulation were not accompanied by proportional increases of post-tetanic potentiation of tension. In other words, maximal potentiation was attained by three to five seconds of tetanic stimulation. The contraction time and the relaxation time in post-tetanic twitches were either less than or about equal to those seen in pretetanic twitches up to the point of maximal potentiation. Prolongation of the period of tetanic stimulation beyond the point of maximal potentiation induced greater contraction times and slower relaxation rates in post-tetanic responses than those found in pretetanic responses.

Post-tetanic responses of muscle 30 minutes after intraperitoneal injection of KCl showed further decreases in height and increases in duration of action potentials. The peak twitch tension attained by tetanus after KCl treatment was greater than that attained by tetanus alone. No evidence of repetition was seen in post-tetanic responses of normal or of KCl-treated rats.

Effects of treppe. There is no significant change in either height or duration of action potentials in responses potentiated by single shocks at one per second. Brief periods of tetanic stimulation at the point of maximal potentiation of tension resulting from treppe brought about further increases of tension in the post-tetanic responses to single shocks. The action potential records of these post-tetanic responses showed increased duration and decreased height.

Muscles potentiatied by intraperitoneal injection of KCl showed increases of twitch tension during treppe which were about equal to the increases seen in treppe of normal muscle. Since the muscle was already potentiated by K before the beginning of exercise the tension attained during treppe in KCl-treated muscle exceeded that obtained by treppe in untreated animals. The action potentials in the KCl-treated muscle underwent little change during treppe. It should be noted that they were already reduced in height and increased in duration before exercise was begun.

<div align="center">DISCUSSION</div>

The findings in this study confirm the observations by Brown and Euler (10) and Feng and Li (11) that marked potentiation of muscle response may be obtained by KCl treatment in the absence of repetitive discharges. The twitching which may occur during close intra-arterial injection of KCl (12, 13) is independent of the potentiated twitch responses to single shocks. The observation that the preponderance of K excess is extracellular throughout the period of KCl treatment (tables 1 and 2) suggests that action of K on the membrane of the muscle fibers may play an important rôle in bringing about 1) increased duration and decreased height of the action potential, 2) increased contraction time and 3) increased twitch tension in response to single shocks.

The changes in positions of the multiple peaks in the action potential, recorded in figure 2A and shown diagrammatically in figure 2B, indicate that dispersion in time of these components contributes to the change in form of the action potential during the progress of KCl treatment. Two factors may be involved in the temporal dispersion of fiber responses in indirectly stimulated muscle: *a*) unequal delay in initiation of response of different muscle fibers and *b*) decreased conduction rate. The increase of the latent period between the stimulus and the beginning of the action potential showed that the start of the response of all fibers is delayed by KCl treatment. The delayed beginning of the endplate potential in frog muscle treated with KCl (14) suggests that the increased latent period between stimulus and action potential is due to an action of K at the neuromuscular junction. It is obvious that the height would be reduced and the duration would be increased in the composite action potential to the extent that the delay of response of the component muscle fibers is unequal. The appearance of multiple peaks in the action potentials of muscle potentiated with KCl (fig. 1B) is regarded as evidence that the response of a part of the muscle fibers is delayed more than that of other fibers.

Granting that the conduction distance between points of excitation and points of recording is unequal in the various muscle fibers, a decrease of conduction rate would result in dispersion of the components of the action potential and thus bring about a decrease in height and an increase in duration of the composite action potential. Recently, Brown *et al.* (15) have shown that addition of adrenalin to a bath containing a phrenic-diaphragm preparation of the rat induced an increase in duration of the muscle action potential, which they attributed to a delay of the spread of the excitation wave in the individual muscle fibers. An earlier report (16) is of particular interest in connection with the present study because it was shown that the potentiating effect of adrenalin on the diaphragm preparation is dependent upon reduction of the K content of the bath fluid.

On the basis of the discussion thus far no change in the height or the duration of the gross action potential would be expected in the absence of dispersion of the components contributing to the action potential. However, two possible effects of K remain which might alter the form of the action potential independently of dispersion. A decrease in rate of depolarization and repolarization would increase the duration of the action potential. Furthermore, a decrease of the resting potential by K excess, as has been shown by many reports, could conceivably result in a decrease in height.

With the exclusion of repetition as a factor only two effects remain which may account for the increased contraction time of K potentiated twitch responses: *a*) dispersion in time of the response of the different fibers and *b*) prolongation of contraction time of the individual fibers contained in the muscle. Moreover, to account for the increase of twitch tension resulting from KCl treatment two assumptions may be made: *a*) the contraction time of the muscle is increased and *b*) the contractile strength of the muscle fibers is increased. Obviously any dispersion of response of component muscle fibers would result in a decrease of twitch tension provided the contraction time of individual fibers remained constant. On the other hand, any increase of contraction time of the individual muscle fibers would bring about an increase of twitch tension, provided a given amount of dispersion existed, because the fibers responding early would allow more effective summation with the contraction of fibers responding late.

The view that K increases the contraction time of muscle fibers is attractive because it offers an explanation, at least in part, for the observed increase both of tension and of contraction time in the potentiated twitch. Furthermore, this view lends itself to the implication of an extracellular action of K on the membrane. Kuffler, (17) has shown that K and veratrine contractures last for the duration of the membrane changes which they induce. He suggested that the essential condition for initiation of processes in the contractile mechanism is the 'removal' of at least a part of the membrane and that any process restoring the membrane will also cause relaxation. The duration of membrane 'breakdown' during a single response of a KCl-treated muscle may be regarded as increased, if it is assumed that conduction is slowed and that the depolarized area is not decreased. Any increase of the depolarized area would further increase the duration of membrane 'breakdown'. If the interpretations regarding contractures are extended to apply to contractions, it is plausible to suggest that the contraction time of a muscle fiber may be increased by increased duration of membrane 'breakdown' resulting from passage of the excitation wave.

The author is not aware of any experimental evidence to support the suggestion by Brown and Euler (10) and Walker (1) that at least a part of the potentiating effect of K on twitch tension is due to increased contractile strength, presumably involving an action of K on the muscle fiber substance.   The present study does not rule out intracellular action of K but it does suggest an extracellular potentiating effect.

The above observations, that in KCl-treated muscle there is increase of twitch tension, increase of twitch peak time and increase in duration of action potential, are readily brought into harmony with the hypothesis offered by Gilson *et al.* (18).   Their treatment of the tension-time course for the twitch assumed instantaneous mobilization of an increment of material as a first step in a series of chain reactions.   If activation of KCl-treated muscles is such that the process of activation or mobilization is not instantaneous but occurs over a significant period of time and, at least for purposes of argument, proceeds at constant rate, there would result an increase of contraction time and an increase of peak tension for the response to a single shock. The form of the twitch response would thus resemble that of a very brief tetanus.

Fenn and Cobb (19) found that tetanic stimulation brings about a decrease of K in the stimulated muscle of the rat.   It was later shown (20) that tetanic stimulation of muscle in the cat produces an increase of plasma K.   The decreased action potential height and increased action potential duration seen after tetanus (fig. 5) offered additional support for the view that a preponderance of K outside of the cells may be important in bringing about these changes in action potential.   Also in this connection it should be pointed out that in a muscle already potentiated by K the characteristic changes of the action potential induced by K are exaggerated by tetanus.

The absence of significant changes of the action potentials during stimulation at one shock per second suggested that at this frequency of response K does not accumulate in appreciable quantity in the extracellular fluid immediately surrounding the muscle fibers.   There is apparently a graded effect upon the action potentials which is dependent upon both frequency and duration of stimulation.   For example, tetanus produced with a stimulus frequency of 200 per second induced a slight decrease of action potential height and no change of action potential duration in post-tetanic responses of muscle stimulated for one to two seconds.   Tetanic stimulation for two to three seconds at the same frequency induced greater decrease of action potential height and some increase of action potential duration (fig. 5A).   Tetanic stimulation for 10 seconds brought about a marked decrease of action potential height and a marked increase of action potential duration (fig. 5B).

The prolonged muscle action potential in adrenalectomized rats (fig. 4A) confirmed the earlier findings (1), but they did not support the view that repetition is involved with the large mechanical responses to single shocks in these animals because none of the leads employed showed any evidence of repetitive discharges distinguishable from the initial action potential.   It is now believed that the changes found in the muscle action potentials of adrenalectomized rats may be due, at least in part, to increases of the intracellular and extracellular concentration of K.   Increased mechanical response to single shocks is perhaps related to changes in concentration of K.

SUMMARY AND CONCLUSIONS

Action potential records with various positions of the lead electrodes were made in muscle with twitch responses potentiated by KCl treatment, adrenalectomy,

tetanus and treppe. The records of action potential showed no evidence of repetition in any of the potentiated responses although the increased peak twitch tension in muscle of KCl-treated and adrenalectomized animals was accompanied by increased contraction time. KCl treatment and tetanus induced a decrease in height and an increase in duration of muscle action potentials. Adrenalectomy brought about an increase in duration of action potential records. Because the height of the action potential is dependent upon the number of muscle fibers contributing to the action potential no conclusions were drawn with regard to height, although the height was usually smaller when compared with action potentials of normal muscle having similarly placed leads. No significant change in the action potential records was found in responses potentiated by treppe induced by single shocks at 1 sec.

Possible effects of K on asynchrony of response, on conduction rate and on the rate of depolarization and repolarization were considered. The relation of these effects to changes in the action potential records was discussed. It was suggested that an increase of extracellular K may play an important rôle in bringing about the decrease in height and the increase in duration of the muscle action potentials. It was pointed out that the slight increase of intracellular K in the KCl-treated muscle is accompanied by a much greater increase of extracellular K. It was further noted that tetanus increases plasma K (20) and that KCl treatment induces changes in the muscle action potential similar to those seen after tetanus. It was suggested that K excess may increase the duration of membrane 'breakdown' and thus increase the contraction time and the strength of contraction of muscle fibers. The possibility that an intracellular action of K in excess might increase the contractile strength of the muscle fiber substance was not excluded.

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BRAIN METABOLISM IN EMOTIONAL EXCITEMENT AND IN SLEEP

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EVIDENCE has been obtained that the metabolic activity of the brain is increased in convulsions, trauma, anoxia and shock. This is indicated by the rise in lactic acid and decrease in high-energy phosphorus compounds found in the brains of animals killed under these conditions. Conversely, there is a fall in the lactic acid in the brain and an increase in labile phosphate in animals killed under anesthesia (1–3). The present investigation was carried out to test if evidence could be found of similar biochemical changes in the brain in sleep and in emotional excitement under normal physiological conditions.

METHODS

Lactic acid estimations were carried out on the whole brains of young Wistar albino rats of 30 to 40 gm. The animals were killed by immersion in liquid air, which produced a rapid fixation of any biochemical changes in the tissues and limited the formation of lactic acid in the brain by post-mortem glycolysis.

Method of fixing with liquid air. Stone (1) found it took 2 to 3 seconds to freeze the brain of a mouse, but Le Page (3) found it took about 40 seconds to freeze completely with liquid air the abdominal organs of a 300-gm. rat. In view of the rapidity of post-mortem changes in the brain, the rate of freezing was reinvestigated with rats of the size used in the present work. Experiments with a calibrated thermocouple inserted into the cranial cavity of 35-gm. rats showed that the surface of the cortex fell to 0°C. in 4 to 5 seconds on immersion of the whole animal in liquid air. With the thermocouple in the deeper parts of the brain the temperature remained practically stationary at 37°C. until a sudden drop in temperature occurred, bringing it below 0°C. in 9, 20, 9 and 16 seconds in four consecutive experiments. While it clearly took several seconds for the wave of freezing to spread from the surface to the interior of the brain, it is unlikely that the circulation of the central parts of the brain stopped immediately or that true post-mortem changes occurred in the interior of the brain throughout the whole of this period. This view is supported by the work of Kerr (4), who found that satisfactory phosphagen figures can be obtained by applying liquid air to the skulls of young cats, in which the process of freezing the brain must take much longer. The observed lowering of the brain lactic acid in sleep, which was found in the present investigation, gave further evidence that post-mortem changes during the process of freezing were probably not extensive with the technique employed.

In obtaining normal values the rats were transferred rapidly from their cages into the liquid air, as any delay in this procedure tended to frighten them and caused the brain lactic acid level to be raised.   Killing by decapitation and subsequent freezing of the heads was found to be less satisfactory as it gave much higher lactic acid figures for normal animals: in three experiments the brain lactic acid figures obtained by this method were 43, 37 and 47 mg. per cent when the heads were transferred to liquid air in 2, 3, and 15 seconds respectively after decapitation.   Kerr also found autolytic changes more marked after decapitation than when the brain was frozen in the whole animal.   The reason may be that with the circulation cut off the brain rapidly becomes anoxic and glycolysis proceeds in the interior during the period of freezing through from the surface.

Lactic acid estimation.   After careful dissection the frozen brains were powdered in a previously cooled crusher and the powder was transferred to weighed centrifuge tubes containing 4 ml. zinc sulphate solution at 0°C. as described by Blatherwick, *et al*. (5).   The method of estimation was that of Friedemann, Cotonio and Shaffer (6), with the modifications and improvements introduced by Friedemann and Graeser (7), Edwards (8) and Stone (1).   Although more laborious, this method was found to give more accurate and more reliable figures than the colorimetric methods of Miller and Muntz (9) and Barker and Summerson (10).   The distillation apparatus was similar to that used by Stone (1), but made all in one piece and therefore containing no joints, tap-grease or rubber connections.   Talc powder was found more satisfactory than glass beads to prevent bumping.   The use of the copper hydroxide precipitation made the method very specific for lactic acid and recovery tests with a standard solution of recrystallized lithium lactate, which were repeated with each experimental series, gave recoveries within 3 per cent of the theoretical with quantities down to 0.1 mg. lactic acid.

<div align="center">RESULTS</div>

Normal series.   In a preliminary series of 10 normal rats taken in the resting state the brain lactic acid level ranged from 13.4 to 24.4 mg. per cent with a mean of 18.8 (table 1).   This figure agreed with the mean of 18.9 mg. per cent for mouse brain found by Stone (1), who also found a wide range of individual values ranging from 12 to 23 mg. per cent.

Brain lactic acid in sleep.   The animals comprising the normal series were mainly littermates of almost identical size and weight and in looking for an explanation for the wide individual variation in the brain lactic acid level, it was noted that lower values were generally given by animals which were dozing at the time when they were transferred to the liquid air, while higher values were obtained with animals which were wide awake.   The investigation of the brain lactic acid in sleeping animals met with considerable difficulty until it was found that deep sleep could be induced by leaving the rats for a time in strong sunlight.   Artificial sunlight was equally effective. Dozing animals opened their eyes at once when touched, but animals in deep natural sleep did not open their eyes or appear to wake up in the brief period of less than a second required to transfer them from their cages into liquid air.   This was therefore taken as the criterion of sleep.   A series of 6 rats taken in the sleeping state showed

less individual variation in the brain lactic acid and a mean level of 12.2 mg. per cent, which was considerably lower than that of the normal series. The difference was statistically significant (P <0.01) when tested by Fisher's 't' test (table 2). The mean for sleeping animals approached the mean of 9.8 mg. per cent lactic acid found for a group of rats lightly anesthetized for a period of 8 to 30 minutes with nembutal.

TABLE 1. LACTIC ACID CONTENT OF BRAIN IN NORMAL RATS

RAT NO.	WT. OF RAT	LACTIC ACID	REMARKS
	gm.	*mg. %*	
1	36	16.2	Dozing
2	38	16.7	Dozing
3	40	20.1	Awake; resisted handling
4	48	17.9	Resting; slight movement
5	43	24.4	Quiet
6	31	22.6	Moving
7	31	22.3	Awake; resisted handling
8	25	15.5	Dozing
9	23	13.4	Quiet
10	40	19.1	Quiet; resisted handling
MEAN..................		18.8	

Recovery of lithium lactate standard 98%.

TABLE 2. LACTIC ACID CONTENT OF RAT BRAIN IN SLEEP AND IN ANESTHESIA

A. SLEEP		B. ANESTHESIA		
Rat no.	Lactic acid	Rat no.	Period of anesthesia	Lactic acid
	mg. %		*min.*	*mg. %*
1	12.3	7	8	13.2
2	10.3	8	8	7.7
3	11.9	9	15	8.0
4	10.8	10	15	4.8
5	13.9	11	30	15.4
6	14.1			
Mean.........	12.2			9.8

Recovery of lithium lactate standard 98%. Brain lactic acid in normal rat included in this series 17.5 mg. %. Period of sleep about 30 min. Anesthesia obtained by intraperitoneal injection of nembutal 50 mg/kg.

Effect of emotional excitement. Of the various methods of producing emotional excitement which were tried, the simplest was that of repeatedly removing their support by allowing them to drop from side to side in a glass beaker. There was a good deal of individual variation in their response to this treatment and some animals were less disturbed by it than others. Often there was a latent period of up to half a minute in which they showed little reaction, but finally they all gave objective evidence of fear, as by urinating, defecating and looking frightened. A few of them

made vigorous muscular movements and tried to jump out of the vessel, but more often they remained perfectly still and gave up trying to right themselves, so that little muscular exercise was involved.   There was probably an element of anger as well as fear in their emotional state, for some of the rats were ready to bite when in this condition; but it may be doubted whether the emotional reactions of the rat can be accurately described in terms which are mainly applicable to man.

Estimations of the lactic acid content of the brains of rats taken after being frightened for $1\frac{1}{2}$ to 4 minutes by the method described gave consistently higher values than those of normal littermate controls which were examined at the same time.   In

TABLE 3.   EFFECT OF EMOTIONAL EXCITEMENT AND EXERCISE ON THE LACTIC ACID
CONTENT OF THE RAT BRAIN

A. FRIGHTENED		B. FRIGHTENED AFTER TUBOCURARINE		C. MUSCULAR EXERCISE	
Rat no.	Lactic acid	Rat no.	Lactic acid	Rat no.	Lactic acid
	mg. %		mg. %		mg. %
1	47.2	12	34.5	17	13.9
2	24.2	13	36.5	18	15.2
3	45.7	14	29.4	19	13.9
4	36.7	15	37.4	20	15.0
5	40.2	16	40.7	21	15.4
6	34.8			22	13.0
7	34.0				
8	23.7				
9	32.2				
10	50.3				
11	43.5				
Mean.......	37.5		35.5		14.4

a) Period of frightening $1\frac{1}{2}$ to 4 min.   *b)* Rats given tubocurarine and after about 5 min., when the muscles were relaxed, frightened for 2 to 3 min.   The blood lactic acid in 3 rats decapitated after treating them in the same manner was 17.8, 22.8 and 18.8 mg.%.   All blood samples were taken from the carotid artery after decapitation.   *c)* Exercise was vigorous running for 4 min.   Recovery of lithium lactate standard 99%.

the frightened animals the lactic acid content ranged from 24.2 to 50.3 mg. per cent with a mean level of 37.5 for a series of 11 animals.   The difference was statistically significant (P <0.01), (table 3).

Experiments in which the period of emotional excitement was varied showed that the rise in brain lactic acid in emotional excitement was a rapid process (fig. 1), which must correspond to a relatively high rate of metabolic activity in the brain.   When the excitation was discontinued, the brain lactic acid soon came back to normal again and normal values were generally obtained within five minutes after discontinuing the stimulus.   The rise in the brain lactic acid appeared to be a transient effect and there was a good deal of individual variation in the rate of rise and fall, as some animals were more easily frightened and remained frightened longer than others.

Effect of muscular exercise.   As far as could be judged from simple observation, the rise in brain lactic acid in the previous experiments corresponded closely with the

degree of emotional excitement and showed no relation to the muscular activity observed in a number of the animals; but since it is known that muscular exercise can cause a rise in the blood lactic acid level, the effect of muscular exercise on the brain lactic acid required careful investigation. The blood lactic acid level is 12 to 18 mg. per cent in the normal rat. In 3 rats taken after four minutes of emotional excitement the blood lactic acid was found to have risen to 52, 47 and 67 mg. per cent; but, assuming the blood content of the brain to be approximately 5 per cent, the blood
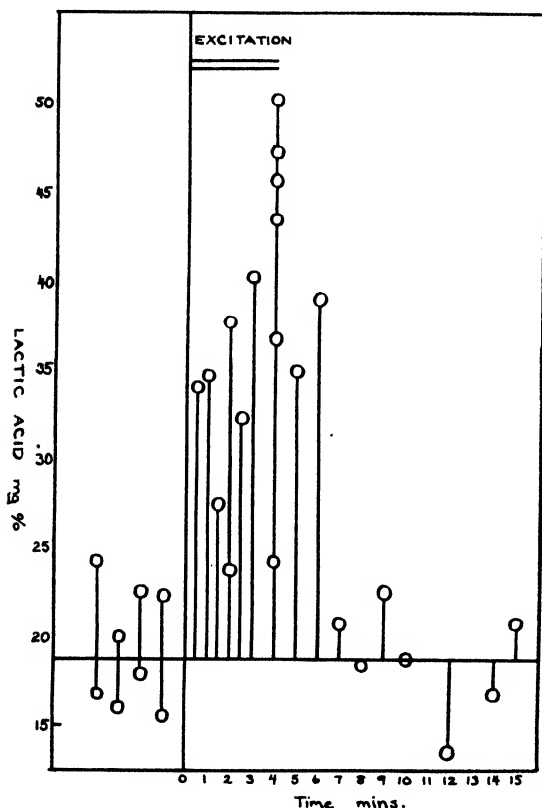
Fig. 1. SHOWING EFFECT OF EMOTIONAL EXCITEMENT on the brain lactic acid. The points on the left of the diagram are normal values, the horizontal line at 18.8 mg. % giving the normal mean. The points on the right of the diagram give lactic acid values for rats sacrificed at the times given on the time scale. The method of frightening was continued for 4 min., except for animals killed after a shorter period of frightening.

lactic acid could not nearly account for the mean level of 37.5 mg. per cent found in the brain. It was unlikely in any case that the brain lactic acid could have come from the blood, since the blood-brain barrier is relatively impervious to anions and it has been shown by Dameshek and Myerson (11) and by Stone (1) that lactic acid injected into the blood stream is not taken up significantly by the brain. This has recently been confirmed by Klein and Olsen (12), who showed in addition that the brain lactic acid is not increased by intravenous glucose, so that it was unlikely that emotional hyperglycemia had played any part. It was concluded that the brain lactic acid was formed in the brain and had not come from the blood.

The view that the rise in lactic acid in the brain in emotional excitement was not attributable to muscular activity was confirmed in a series of experiments in which

rats were excited after administering tubocurarine, so that muscular activity was practically abolished. The effective dose for this purpose was 0.1 ml. of a solution of 0.1 mg/ml. d-tubocurarine chloride per 40-gm. rat, given intraperitoneally. With this dose the respiration was not unduly embarrassed and the animals were not cyanosed, but muscular activity was greatly diminished. Emotional excitement produced the same rise in brain lactic acid in these animals, the figures ranging from 29.4 to 40.7 with a mean of 35.5 mg. per cent, although the blood lactic acid remained almost in the normal range (table 3). It therefore appeared that the rise in lactic acid in the brain in emotional excitement could not be attributed to the accompanying muscular activity.

In a series of further experiments designed to test directly the effect of muscular exercise alone on the brain lactic acid it was necessary to take special precautions to avoid emotional excitement, for it is hardly possible to induce untrained animals to take vigorous muscular exercise without exciting them. With this object in view a series of young rats weighing about 15 gm. were slowly conditioned to running for periods up to four minutes twice daily on an exercising wheel. After training in this way for eight days, by which time they weighed about 35 gm. they took their exercise without showing any signs of anxiety and indeed they appeared to enjoy it. Brain lactic acid estimations on a series of 6 trained rats after a period of four minutes vigorous running gave figures ranging from 13.0 to 15.2 with a mean of 14.4 mg. per cent. There was thus no evidence of any rise in the brain lactic acid in muscular exercise: the mean level after exercise was even somewhat below that of the norma series. This may be due to the effect of frequent handling in reducing their anxiety when handled.

DISCUSSION

It has been shown that the lactic acid content of the rat brain, analyzed after rapid fixation by freezing in liquid air, depends on the physiological state of the animal at the time. Rats taken in the sleeping state gave a significantly lower brain lactic acid content than controls in the normal waking state. Rats taken during emotional excitement gave a brain lactic acid level considerably higher than the normal and 300 per cent above the mean level for sleeping animals. Unless it is believed that these changes occurred in the brief period of freezing with liquid air, it must be concluded that they represent biochemical changes occurring in the brain *in vivo* under normal physiological conditions.

Muscular exercise appeared to play no part in the rise in lactic acid in the brain in emotion since *a*) the effect was still observed in animals immobilized by tubocurarine and *b*) no rise in the brain lactic acid occurred in muscular exercise alone without emotional excitement. The lactic acid content of the brain is not increased by adrenaline injection (1, 12) and the rise in lactic acid in the brain in emotion was observed in the absence of any significant rise of lactic acid in the blood. The simplest explanation of these observations is that in emotional excitement the increased functional activity of the brain is associated with increased glycolytic activity, involving a breakdown of high energy phosphorus compounds and the liberation of lactic acid. The changes in the brain would thus parallel those which occur in func-

tional activity in muscle. This view is supported by the work of Stone (1) and Le Page (3) on the changes in the brain produced by anesthetic and convulsant drugs. That biochemical changes occur in the brain in emotion is indicated by the changes in the electroencephalogram, as also by the chromatolysis in the nerve-cells in prolonged emotional excitement.

The results of the present investigation are in general agreement with the observations of Stone and of Le Page, but they suggest that the rise in brain lactic acid in exercise observed by Stone should be attributed rather to the emotional excitement, which is hardly avoidable when untrained animals are made to take strenuous exercise. Emotional excitement may contribute to the biochemical changes in the brain produced in some cases by drugs and in experiments such as those of Le Page on experimental shock. Gibbs *et al.* (14) reported that the brain normally liberates a small but significant amount of lactic acid into the blood, as shown by arterio-venous differences in experiments on man. This gives evidence of the formation of lactic acid in the brain under normal physiological conditions. The present work suggests that the rate of formation of lactic acid varies with the functional activity of the brain, being lowered in·sleep and increased in emotional excitement.

SUMMARY

The lactic acid content of the rat brain is reduced in sleep and increased in emotional excitement. The rise in lactic acid in the brain in emotion is not due to concomitant muscular activity, since the effect was still observed in animals immobilized by tubocurarine. The brain lactic acid was not raised by muscular exercise in trained animals. The rise in lactic acid in the brain in emotion is a transient effect, followed by a rapid return to normal when the stimulus is discontinued.

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INTRACRANIAL NOVOCAIN ANESTHESIA IN FROGS

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RECENTLY a method of anesthetizing fish (1) by the injection of 0.25 to 1.0 cc. of a 3 to 7 per cent novocain solution into the cranial cavity was described. Fish treated in this way are immobilized after a short excitation stage; the effect of the injection lasts from 30 to 60 minutes. They continue to breathe and if the head is kept submerged in a small seawater tank the heart can be used for a long time for experiments.

The usual way to immobilize or anesthetize frogs is either to use curare or to use the method of crushing the brain and spinal cord. This procedure causes a variable loss of blood. Therefore the use of intracranial novocain injections (1) was tried for frog experiments too. The method proved as effective here as in fish. For the experiments big bullfrogs were used, weighing between 250 and 500 gm. The solution of procaine hydrochloride (Novocain) used was between 3 and 5 per cent in strength. From 0.4 to 0.6 cc. of the solution was injected. The skull of frogs is harder to pierce than that of even a big selachian. Therefore somewhat stronger needles (20-gauge) were used. The preferred place for introducing the needle into the skull is caudally of the middle of a line connecting both eyes. The injection can also be made through the palate. In both methods the needle remained in some experiments in its place up to the end of the experiment to make sure afterwards that its apex was really in the cranial cavity.

Within a few minutes the animal was anesthetized and immobilized after a short time of excitation. About an hour later the animal recovered. If brought back into the basin it was in each instance found to behave normally the next morning and the following days. The experiment could be repeated on the same animal on the next day. It may be open to discussion whether the fading of the procaine effects is due to reabsorption and elimination, or to destruction of novocain by a procaine esterase. Up to the present time there has been no proof of the existence of a procaine esterase in frogs. In a previous paper, however, it was shown that the blood of turtles contains an amount of procaine esterase, the strength of which is between that of a rabbit and a cat (2).

OBSERVATIONS ON FROGS ANESTHETIZED WITH INTRACRANIAL NOVOCAIN INJECTIONS

A few observations made on such animals in procaine narcosis may be mentioned. The first effect as a rule is a short excitation stage similar to that found in fish. At the beginning of the immobilization, leg reflexes could still be elicited. Pinching or pressing of the foot produced a flexor reflex of the homolateral leg. In deeper

narcosis sometimes also only a flexor reflex of the contralateral leg followed the stimulus. Even at a time when these reflexes could no longer be provoked in the frog suspended by both arms or lying on its back, the legs were always brought spontaneously to their normal position, if the frog was put on its belly. That happened spontaneously as well as in the way of a reflex after pinching a leg. At a time when the leg reflexes were still present, they were easily exhaustible (fatigue) and in some instance if tried two or three times in quick sequence they were no longer elicited. After a minute of rest they could again be produced.

The fact that the occurrence of these reflexes depends on the position of the animal could be shown in various ways. At a certain stage of anesthesia the leg reflexes could not be produced if the animal was lying on its back with its legs on the table, but could be produced easily if the animal was lying on its back as before but with the leg hanging over the edge of the table. Another reflex, easy to produce, also proved to be dependent upon the animal's position. If the animal were lying on its back it was possible by tapping its belly or its flanks or by lightly touching the ventral part of the upper thighs to provoke a croak reflex. This reflex could not be produced if the frog were lying on its belly.

In a former paper it was mentioned that fish narcotized by intracranial novocain injections showed at the beginning or the end of the narcosis a nystagmus (1) as previously seen in rabbits (3) during intravenous nembutal narcosis. In none of the frogs could anything similar to real nystagmus be seen. When the frogs recovered from narcosis, however, the following behavior could be observed. The eyes were rhythmically protruded and retracted and whenever the bulbi were protruded, the eyelids were opened wide, the nostrils closed and the flanks drawn in. This eye movement could either appear spontaneously or be provoked by touching an eye of the animal or its belly. It resulted sometimes in groups of such rhythmic contractions lasting a few minutes.

SUMMARY

A method is described for immobilizing and anesthetizing frogs by intracranial injection of approximately 0.5 cc. of a 3 to 5 per cent solution of novocain. The anesthesia which follows a short stage of excitation lasts about one hour. It is followed by complete recovery.

During the anesthesia the behavior of reflexes and the influence of the position of the animal on leg reflexes and croak reflexes were studied. During the time of recovery a kind of nystagmus equivalent appeared spontaneously, or it could be provoked as a reflex by touching the eyeball or the belly.

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DESCENDING NERVE FIBERS SUBSERVING HEAT MAINTENANCE FUNCTIONS COURSING WITH THE CEREBRO-SPINAL TRACTS THROUGH THE PONS[1]

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DURING a study concerned with determining whether any of the nerve fibers subserving heat maintenance functions decussate at the midbrain or pontine levels (1), we were led to suspect that some of these fibers descend from the hypothalamus in close association with the cerebrospinal tracts. The experiments described in this paper were designed to test this probability.

METHODS

Operative. The dorsal aspect of the inferior colliculus was exposed by elevation and retraction of the occipital lobe. A small rigid probe was projected downward along the midline until the base of the skull was reached, then withdrawn slightly, after which it was pulled laterally through the soft tissue of the stem. In this way a hemisection of the stem was accomplished except for sparing a small amount of tissue ventrally and medially. The same procedure was subsequently carried out on the opposite side. The end result was a complete transection of the dorsal aspects of the brain stem, leaving, from preparation to preparation, a varying amount of ventral tissue unsevered. It was calculated that in certain instances the only descending fibers left unsevered would be those in the pyramidal bundles (*dog 76*, fig. 1).

Postoperative Management. The animals were maintained in 28 to 30° C. incubators postoperatively until there was evidence (raised rectal temperature and shivering) that some heat maintenance functions were retained. They were then removed to hammocks at ordinary room temperature (24° to 26° C.). In the event no heat maintenance ability became evident, they were maintained continually in 28° to 30° C. incubators. On the morning after operation (20 hours postoperative), the animal's heat maintenance powers were assayed by placing it in an environment of 3° to 10° C. for a 6- or 8-hour period as previously described (2). After this test the animals were placed on a hammock in an environmental temperature of 24° to 26° C. and the rectal temperature followed for several hours. Some preparations were terminated on the second day after operation, while others were continued for several days. These latter were usually terminated by spontaneous death of the animals.

Tissue: Method of Determining Extent of Lesion. The animals which were terminated were done so under full sodium pentobarbital anesthesia by opening the thorax and cutting the superior vena cava. It was then perfused through the left heart with 0.9 per cent saline solution followed with 10 per cent neutral formalin. The cadaver was placed in a refrigerated box for several hours to allow for maximal hardening of the central nervous system. The brain was then removed, blocked and trimmed, and returned to formalin for a period of 24 hours. The extent of the lesion was determined *a*) by careful gross inspection after removal of the pia and *b*) by cutting through the unsevered tissue in a transverse direction or by cutting the brain stem sagittally along the midline.

In the animals which were found dead, the brain was removed carefully and placed in 10 per cent neutral formalin for 24 hours, at which time the pia was removed and the block containing the

lesion was cut out and trimmed appropriately. This block was returned to 10 per cent formalin for an additional 24 or 48 hours.

It was found that we could routinely section the greater part of the dorsal brain stem tissue at the level of the pons without materially affecting the animal's ability to combat a cold environment. There was some variable deviation from the normal in the heat maintenance powers in these animals, as shown by a mild drop in rectal temperature during the early part of the cold environment test and an overshooting of rectal temperature with a continuation of shivering beyond the termination of the test. They always, however, combatted successfully an environmental temperature of 5° to 10°C. for 6 to 8 hours, during which time the rectal
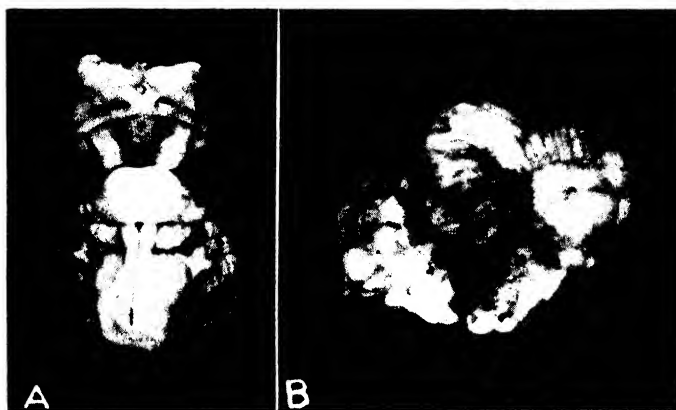
Fig. 1.   *A*. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 76*, showing the level and extent of transection. *B*. Photograph of cross-section of caudal portion of brain stem after unsevered portion of pyramidal bundles was cut through with a razor blade. It is to be noted that the dorsal one third or one half of pyramidal bundles was severed by the operative procedure.

temperature fell at the most 2°C., with prominent generalized shivering throughout the test.

The record for the 6-hour 5°C. test on *dog 85* is shown in figure 2C. It is to be noted that the deviation from the normal in this animal consisted of *a*) a drop in rectal temperature from 39.4°C. to 38°C., which was regained during the test, *b*) the abrupt rise of the rectal temperature to 41°C. on cessation of the test, and *c*) a continuation of generalized shivering for several hours after the cessation of the test. It is to be noted from the photograph in figure 2A that the lesion cut through the lateral aspects of the pons, but that the entire medial portion of the pons remained undisturbed. The photograph in figure 2B shows the amount of the ventral tissue of the stem which remained unsevered. This can be seen to be the tissue of the pons proper.

It was also found, on the basis of several successful experiments, that we could routinely cut all the tissue of the brain stem except for the pyramidal bundles as they become exteriorized at the caudal extent of the pons, without eliminating all heat maintenance powers. In these instances there was a greater deficit during the test
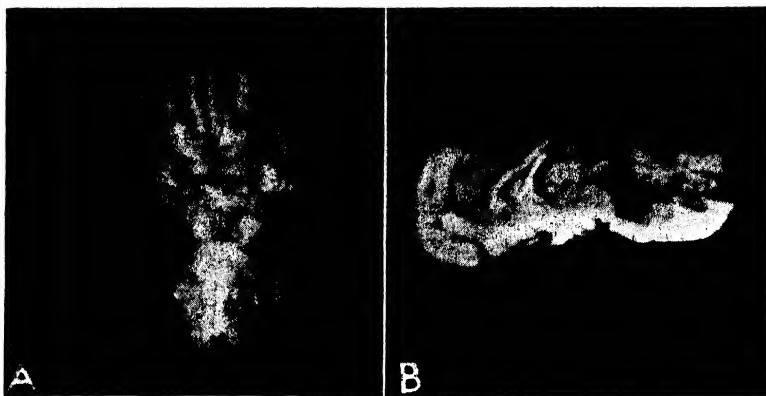
Fig. 2. *A*. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 85*, showing level and lateral extent of transection. It is to be noted that the lesion was symmetrical on both sides and involved only the lateral aspect of the pons ventrally. *B*. Photograph of medial aspect of right half of brain stem of *dog 85*, showing level and extent of transection. It is to be noted that a slight amount of the tissue immediately dorsal to the pons remained unsevered. *C*. Graph of cold environmental temperature tests on *dogs 82, 85* and *88*. See text for further details of these animals.
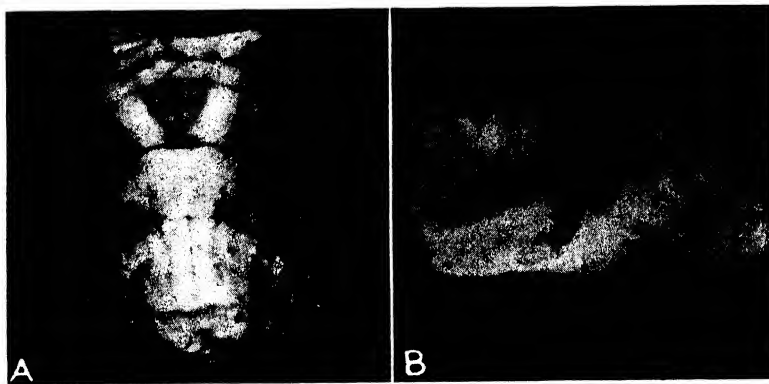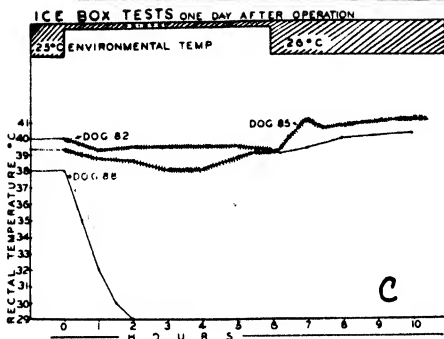
Fig. 3. *A*. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 79*, showing level and extent of transection. Note that all of the tissue was transected at the caudal level of the pons, except for the pyramidal bundles. *B*. Photograph of medial aspect of left half of brain stem of *dog 79*, showing level and extent of transection. It is to be noted that all of the tissue at this level was completely transected except for the pyramidal bundles. For a short distance immediately dorsal to the pyramidal fibers, the lesion is not filled with blood clot and can be seen only by careful inspection as a thin tissue defect. *C*. Graph of cold environmental temperature tests on *dogs 79, 82* and *88*.
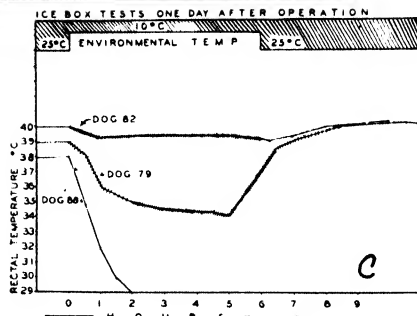
than in the former group, but considerable maintenance powers were always in evidence. These features were well shown in the experiment on *dog 79*, the results of which are illustrated in figure 3.

This dog's rectal temperature on the morning after operation was 39°C., after being housed during the night on a hammock in a room at 25°C. When placed in a 10°C. environmental temperature, the animal immediately began atypical shivering (slight and periodic), and the rectal temperature fell precipitantly. After two hours, continuous vigorous shivering began, and the rate of fall in rectal temperature decreased accordingly.

In addition, it was found that considerable gross encroachment upon the dorsal aspects of the pyramidal bundles was compatible with the sparing of considerable heat maintenance powers. This was evident in *dog 76*, the extent of the transection being illustrated in figure 1. Although this dog died before a cold environmental test was run, it maintained a rectal temperature of 38.5°C. when housed on a hammock in a room at 25°C., and under these conditions exhibited typical generalized shivering.

If the transection was complete, the animal exhibited no heat maintenance abilities (3). The response of a dog (*dog 88*), which had a complete transection except for a few strands of pyramidal fibers, illustrates this fact and is reproduced in both figures. This animal showed no evidence of any maintenance powers during the cold box test on the day after operation; however, it did possess remnantal powers as shown by a 'regulation interval' ranging from 10°C. the day after operation to 13°C. on the tenth postoperative day.

The responses of the above described animals to the cold environment test are contrasted in figures 2 and 3 with that of *dog 82*, which had the pons hemisected on the right side the day previous to the test. This dog exhibited a mild postoperative hyperthermia which frequently follows a major traumatic lesion at the level of the pons, but responded normally to the cold environment test.

<div align="center">DISCUSSION</div>

Although the foregoing experiments were entirely acute in type, they clearly demonstrate that some thermogenic fibers, both shivering and nonshivering, descend as a component part of the pyramidal bundles at the level of the pons.

The experiments do not give any reliable indication as to the relative proportion of the over-all heat-maintenance fibers which descend through the pons with the cerebrospinal fibers. The fact that there was some deficit in heat maintenance powers in these animals does not necessarily indicate that the number of heat maintenance fibers coursing in the pyramidal bundles is inadequate to maintain normal maintenance functions, because some of the fibers may have been temporarily defunctioned by the lesion sequelae. This question can be answered only by chronic experiments.

To find thermogenic fibers coursing in the pyramidal bundles is of particular interest for two reasons. First, recent anatomical evidence has demonstrated that fibers other than those taking origin from the pyramidal or Betz cells course with the cerebrospinal tracts, many of which conform to the conventional anatomical

criteria for classifying them as autonomic fibers (4).   Second, clinically vasomotor and related autonomic disturbances have long been observed on occasion to be associated with intracranial crises localized to or closely associated with the cerebrospinal tracts (5).

SUMMARY

The entire pons save for sparing the pyramidal bundles was transected in dogs without eliminating heat-maintenance powers.

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EFFECT OF ALLOXAN, PANCREATECTOMY AND ADRENA-LECTOMY ON PLASMA AMINO NITROGEN IN THE DOG AS STUDIED BY MEANS OF HEMORRHAGE[1]

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ENDOCRINE influences in nitrogen metabolism have been investigated largely through studies of changes in N excretion and in the NPN level of the blood, produced as a result of hormone injection or removal of the gland concerned. In this manner it has been demonstrated that injection of the growth hormone (1) and of androgens (2) cause N retention, whereas injection of estrogens (3), thyroxine (4) and the adrenal steroids (5) increase N breakdown. From a study of the rate of accumulation of NPN in the blood of nephrectomized dogs, Mirsky (6) concluded that the administration of an anterior pituitary extract which contained the growth hormone induced N retention only when the pancreas was present. Lack of insulin was held responsible for this effect.

Recently, White and Dougherty (7) by analyses of various tissues have secured evidence which indicates that the cortical steroids cause mobilization of N from lymphoid tissue, and that the injection of thyroxine is followed by a removal of N from the carcass.

It is difficult to study the influence of hormones on N metabolism by means of plasma amino N changes because of the remarkable stability of the amino acid level of the blood. However, it has been shown in the rat, that following hemorrhage liver function is depressed (8), and the blood amino N level rises (9). A rise in the arterial amino N level and a significant femoral arterio-venous amino N difference, indicating the liberation of amino acids from the muscles of the leg, were demonstrated after hemorrhage in the dog (10).

In this paper are reported the arterial plasma amino N levels and the femoral A-V amino N differences in *a*) alloxan-treated, *b*) depancreatized and *c*) adrenalectomized dogs before and after a standard hemorrhage.

PROCEDURE

Approximately 55 mgm/kgm. of alloxan were injected into each of a series of dogs. In a few instances additional, larger injections were made if the first dose proved inadequate to produce a sustained hyperglycemia. Only those animals in which the blood glucose remained above 185 mgm per cent were used. The experiments were performed at least three days after the last injection.

The depancreatized animals were maintained with protamine Zn insulinate until the incisions had healed. No supplement was added to the ordinary diet of commercial 'Friskies'. Insulin was withdrawn two to five days before the experiments were performed.

The removal of the adrenal glands was carried out in two stages. Each gland was removed in one sharply delineated mass. After the second operation, the animals were maintained with commercial Doca and given 0.9 per cent saline to drink. The injections of Doca were stopped at least two days before the experiment. The 5 animals upon which successful experiments were carried out ate well and maintained their body weights after operation. Food was withheld for 14 to 24 hours prior to each experiment.

Comparable degrees of hemorrhage were produced without anesthesia by the method of Walcott (11). The animals were bled from, and 25 per cent of the bleeding volumes were immediately returned into, the right femoral artery. In the adrenalectomized dogs, 30 per cent of the bleeding volume was reinfused in an effort to extend their survival periods. None of the animals reported on in this paper survived the hemorrhage.

The procedure for obtaining simultaneous (within two minutes) blood samples from the left femoral artery and vein with minimum disturbance to the circulation has been described elsewhere (10). Blood samples were taken before bleeding and at hourly (half-hourly in the experiments on adrenalectomized animals) intervals until death occurred. Those taken within 20 minutes of death are designated as terminal.

Blood glucose was determined by the method of Somogyi (12) and plasma amino N was estimated by the manometric ninhydrin method (13). All data are presented in terms of mgm/100 cc. of plasma.

<div align="center">RESULTS</div>

Arterial Plasma Amino N. The average control arterial plasma amino N levels of the alloxan-injected, depancreatized and adrenalectomized dogs and the changes in these values after hemorrhage are shown in table 1. The average values which were found previously in a series of otherwise untreated, similarly bled dogs (10) are included for comparison. The survival periods have been divided into quarters, each approximating one hour, except for the adrenalectomized animals in which each quarter of the survival period averaged 20 minutes.

The arterial blood glucose levels of the alloxan-injected dogs averaged 258 mgm. per cent (range 187 to 375) and those of the depancreatized animals, 294 mgm. per cent (range 244 to 385).

1. *Before hemorrhage.* The average control arterial amino N value of 8 alloxan-injected dogs was 4.41±0.45 mgm per cent, and of 8 depancreatized dogs, 7.27±0.73 mgm. per cent, as compared with that of the control series which was 3.65±0.10 mgm. per cent. Statistically, there is a significant difference between each of the three series (p<0.01). Adrenalectomy in 5 dogs caused no change in the arterial level, the values averaging 3.89±0.26 mgm. per cent (table 1).

2. *After hemorrhage.* Both the control and the alloxan-injected animals showed

an increase in the arterial amino N levels after hemorrhage. The two series did not differ significantly from each other as to the magnitude of the increase which occurred. The depancreatized dogs, however, after a similar hemorrhage, showed no significant change in the concentration of arterial amino N. Since the depancreatized dogs had higher control values, the average arterial levels of the normal and alloxan-injected, bled animals approached that of the depancreatized dogs during the course of the hemorrhage. Statistically, the change from the control value of the depancreatized series differs significantly from that of the normal and alloxan series in each quarter of the survival period (p < 0.01).

The adrenalectomized dogs proved so susceptible to stress that it was impossible to obtain survival times longer than one and one half to two hours despite the reinfusion of 30 per cent of their bleeding volumes, whereas only 25 per cent was returned to the dogs in the other series. However, it is evident (table 1) that despite an average survival time of only 80 minutes the arterial level rose in a manner compa-

TABLE 1. ARTERIAL PLASMA AMINO NITROGEN LEVELS (BEFORE AND AFTER HEMORRHAGE)

EXPERIMENT	No. of Dogs	PLASMA AMINO NITROGEN IN MGM. PER 100 CC.					
		Control	Survival Time				
			No. of Dogs	¼	½	¾	Terminal
Normal.....	35	3.65±0.10[1]	16	4.76±0.15	5.16±0.20	5.84±0.20	7.20±0.32
Alloxan[2].....	8	4.41±0.44	8	5.55±0.49	5.49±0.55	6.05±0.66	8.53±0.71
Depancr.[3]....	8	7.27±0.77	5	6.69±0.46	6.54±0.52	6.60±0.56	7.66±0.94
Adrenx......	5	3.89±0.26	5	4.86±0.25	5.54±0.21	6.17±0.25	6.92±0.37

[1] Mean ± standard error of mean
[2] Average blood glucose 258 mgm. per cent (range 187–375).
[3] Average blood glucose 294 mgm. per cent (range 244–385).

rable to that observed in the control, bled dogs whose survival times averaged four hours.

Femoral Arterio-venous Plasma Amino N Differences. The femoral A-V amino N differences of the alloxan-treated, depancreatized and adrenalectomized animals before and after hemorrhage are shown in table 2. In all instances the venous level was higher, that is, amino N was added to the circulating blood as it passed through the leg.

1. *Before hemorrhage.* The A-V amino N differences averaged 0.32±0.19 mgm. per cent for the adrenalectomized, 0.52±0.17 mgm. per cent for the alloxan-injected and 0.82±0.43 mgm. per cent in the depancreatized dogs as compared with 0.18±0.04 mgm. per cent in the normal series (table 2). The difference between the normal and the alloxan series (p=0.03) and between the former and the depancreatized series (p=0.02) is of borderline significance. One of the animals injected with alloxan and two of the pancreatectomized dogs, however, showed a release of amino N into the blood far greater than was ever observed in the control animals. One of the adrenalectomized dogs had a negative A-V difference, that is, the concentration of amino N was less in the blood leaving than in that which entered the leg.

2. *After hemorrhage.* As a result of the type of hemorrhage used in these experiments, the blood flow through the leg in control and diabetic animals decreased to approximately 20 per cent of the pre-hemorrhage value (14). The femoral A-V differences tend to be magnified by the smaller quantity of blood flowing through the legs. After hemorrhage the average A-V difference of the alloxan-injected dogs was approximately one and one half, and that of the depancreatized animals was five times as great as the average value found in the control, bled series (table 2). Statistically, the post-hemorrhage A-V differences of the three series are significantly different from each other ($p < 0.01$).

The adrenalectomized dogs showed no rise in their average A-V differences after hemorrhage, and, indeed, one animal had a negative control A-V difference which became increasingly more negative throughout the survival period.

TABLE 2. FEMORAL ARTERIO-VENOUS AMINO NITROGEN DIFFERENCES (BEFORE AND AFTER HEMORRHAGE)[1]

EXPERIMENT	No. of Dogs	Control	A-V AMINO NITROGEN DIFFERENCES IN MGM. PER 100 CC.				
			Survival Time				
			No. of Dogs	¼	½	¾	Terminal
Normal......	16	0.18±0.04	10	0.51±0.08	0.71±0.12	0.66±0.14	0.45±0.10
Alloxan......	8	0.52±0.17	8	0.97±0.14	1.24±0.20	1.40±0.26	0.79±0.44
Depancr......	5	0.82±0.43	5	2.73±0.72	3.25±0.69	3.01±0.55	3.14±0.81
Adrenx......	4	0.32±0.19	4	0.31±0.25	0.21±0.49	0.20±0.47	0.11±0.33

[1] In all instances the venous levels were higher. See footnotes to table 1.

The blood volumes, bleeding volumes and survival times of the control, alloxan-injected and depancreatized animals were almost identical. In addition, measurements of the blood flow through the femoral vein before and after hemorrhage (14), show remarkable agreement between the three series of animals in the vascular response to the hemorrhage. From these measurements, it would appear that the changes in plasma amino N levels observed may be ascribed to metabolic rather than purely vascular differences between the types of experimental animals used.

DISCUSSION

A significant elevation in the arterial amino N level was found in alloxan-injected and depancreatized animals within one week following the production of hyperglycemia (table 1). The addition of amino N to the blood as it passed through the leg was also greater in diabetic animals than in control dogs (table 2).

The significance of alterations in the plasma amino N level is not known although values above normal are usually associated with liver damage. An elevation in the blood amino N level has been described after experimental pancreatectomy (15), and in human diabetics (16, 17). The latter authors reported that insulin lowered the blood amino N level of their patients. A decrease in the blood amino N level after the injection of insulin into normal animals has also been described (18).

Differences in amino N metabolism between alloxan-injected and depancreatized dogs, in which the extent of hyperglycemia was approximately the same, are indicated by the data obtained. The arterial amino N level of the depancreatized dogs was significantly higher than that of the animals injected with alloxan. A further difference appears in the response to hemorrhage. Whereas the arterial level of the dogs injected with alloxan increased in a manner closely resembling the response obtained in control, bled dogs, hemorrhage produced no significant alteration in the arterial amino N values in animals from which the pancreas had been removed. The changes in the concentration of amino N as the blood passed through the leg likewise reveal significant differences between the two series, the values being greater in the depancreatized animals. Hemorrhage did not alter this relationship; the A-V difference in each series increased proportionately, and the liberation of amino N from the muscles was greater in depancreatized than in alloxan-injected animals.

The failure of a post-hemorrhagic rise in the plasma amino N level to occur in depancreatized dogs cannot be ascribed to increased excretion in the urine, since, following the hemorrhage, the animals were anuric. In the depancreatized dog subjected to the stress of hemorrhage, as compared with intact or alloxan-treated animals under the same conditions, therefore, much larger amounts of amino N were removed from the blood stream by the liver.

The elevated arterial amino N levels of the diabetic animals may be caused by a disturbance of the equilibrium between amino acid production and removal, resulting in a shift of the equilibrium toward a higher plasma amino N level. This 'higher setting of the thermostat', however, is not a reflection of an impaired ability of the liver to remove amino acids because in the depancreatized animals, in which peripheral production was the greatest, the arterial amino N level did not rise after hemorrhage.

A direct toxic effect of alloxan upon the liver may explain the differences in amino N metabolism observed between the alloxan-injected and the depancreatized animals, although those animals which were injected several times with increasing amounts of alloxan showed no difference in response from animals injected only once. It is also possible that in the depancreatized animal deprived of many of its proteolytic enzymes, the mobilization and utilization of endogenous nitrogen sources may proceed at an accelerated rate. Until this possibility is explored, the postulation of a second hormone in the pancreas would not appear to be necessary.

The adrenalectomized animals cannot be compared directly with the other series because of differences in the vascular response and the marked susceptibility of these animals to stress. However, the animals showed amino N changes the magnitude and direction of which are of interest. Thus, the average arterial amino N level of the adrenalectomized dogs did not differ from that of the untreated series either before hemorrhage or in any quarter of the survival period even though their total survival times averaged only 80 minutes as compared with 4 hours for the control, bled series (table 1). The average femoral A-V difference of the adrenalectomized animals, in contrast to the results obtained from control, bled dogs, showed no rise following hemorrhage. This observation lends support to the theory ad-

vanced by Long, Katzin and Fry (19) that the adrenalectomized animal is unable to mobilize its endogenous nitrogen at a normal rate.

Comparison of the rates of peripheral production of amino N after hemorrhage as measured by femoral A-V differences in depancreatized and adrenalectomized animals reveals no correlation between the output of amino N from the muscles and the arterial levels observed. For example, despite the fact that there was no increase in the liberation of amino acids after hemorrhage in adrenalectomized animals, the arterial level increased as much in 80 minutes as it had in 4 hours in normal, bled dogs. On the other hand, depancreatized dogs showed no increase in the arterial level after hemorrhage in spite of a large addition of amino acids from the legs. It would appear, therefore, that the plasma amino N level in these experiments was not dependent upon the rate of production but that regulation of the arterial level was determined by the rate of removal of amino acids from the circulation.

SUMMARY

Arterial plasma amino N levels and femoral arteriovenous amino N differences were determined in alloxan-injected, depancreatized and adrenalectomized dogs before and after a standard hemorrhage, and the values obtained were compared with those from a series of normal, bled animals.

The average control arterial level was elevated in alloxan-injected dogs, 4.41 mgm. per cent, and was much higher in depancreatized animals, 7.27 mgm. per cent, as compared with the control series, 3.65 mgm. per cent (table 1). Following hemorrhage the average arterial level rose in the alloxan-injected and normal animals but showed no change in the animals of the depancreatized series.

The average control femoral A-V amino N differences, which indicate the addition of amino acids to the blood from the muscles of the leg, were higher in alloxan-injected, and much greater in depancreatized, than in normal animals. After hemorrhage the A-V differences increased in each series, maintaining, however, the same proportionate relationship (table 2).

Significant differences between the alloxan-injected and the depancreatized dogs appeared in *a*) the arterial level, *b*) the response in the arterial level after hemorrhage and *c*) the A-V amino N difference.

The average control arterial level of the adrenalectomized dogs and the rise which occurred in each quarter of the survival period after hemorrhage did not differ from that of the animals of the normal series even though their survival times averaged only one-third that of the normal series. The average femoral A-V amino N differences in adrenalectomized dogs showed no increase after hemorrhage.

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ALLOXAN DIABETES IN SHEEP UNDER FASTING AND NON-FASTING CONDITIONS

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INVESTIGATION of the diabetic state in the calf (1, 2) and goat (3) have indicated that the processes regulating carbohydrate metabolism in ruminants differ in certain respects from those of carnivores, from which much of the knowledge of carbohydrate metabolism has been derived. As von Mering and Minkowski (4) showed in 1889, pancreatectomy in the dog results in a syndrome similar to severe diabetes mellitus in man. Subsequent work has shown that hyperglycemia, ketonemia, and marked increases in glucose, ketone, and nitrogen excretion in the urine accompany the clinical signs of polydipsia, polyuria and polyphagia in this group of animals.

In the depancreatized calf, however, Cook and Dye (1) reported a very mild form of diabetes. Hyperglycemia was present when the calf was fed, but hypoglycemia developed when feed was withheld. Greeley (3) found that the depancreatized goat can maintain a healthy state, even without the administration of insulin.

The discovery of Dunn *et al.* (5), that intravenous administration of alloxan monohydrate, a derivative of uric acid, causes necrosis of the insulin-producing cells of the pancreatic islets in rabbits, has opened a new era in research on intermediary metabolism. McCandless and Dye (2) reported low glucose tolerance and glycosuria, but normal blood glucose levels, in a calf treated with alloxan. Alloxan diabetes in goats was found to be moderately severe by Saviano (6). Jarrett (7) observed severe diabetes in sheep treated with alloxan, and a high degree of susceptibility to the toxic effects of the drug. In a preliminary paper from this laboratory (8), studies of 2 alloxan diabetic ewes were reported. The present paper is a continuation of that work.

METHODS

Alloxan diabetes was produced in 8 grade sheep by the intravenous administration of alloxan monohydrate (Eastman), in doses of 125, 100, and 75 mg. per kgm. in *sheep 1, 2,* and *3* through *8*, respectively. The drug was dissolved, immediately before use, as a 5 per cent solution in sterile distilled water. It was then injected rapidly into the jugular vein. Blood samples were taken for glucose determination immediately preceding the injection, and at selected intervals during the first 24 hours. On subsequent days, blood glucose was determined at 8 or 9 a.m. and an aliquot of the 24-hour urine was analyzed for glucose, ketones and nitrogen. Nitrogen determinations could not be carried out on some samples because of fecal contamination. The blood was analyzed for ketones several times in the experimental period of each animal.

After preliminary studies of blood and urine chemistry, all food was withdrawn and the sheep were studied under fasting conditions. It should be pointed out that the ruminant continues to absorb nutrients for several days after the initiation of the fast, as a consequence of the large amount of material in the rumen. Experimental observations in this laboratory indicate that postabsorptive conditions are not attained until the 3rd or 4th day of the fast.

The survival period of each animal was determined. Immediately after death, pieces of pancreas, liver and kidney were removed, fixed both in Bouin's and in Zenker's solutions, and imbedded in paraffin. The tissue sections were stained with hematoxylin and eosin.

Blood and urine glucose was determined by the Somogyi-Shaffer-Hartmann method (9); urine glucose was corrected for non-fermentable reducing substances by the use of a yeast blank. Muscle and liver glycogen was determined by the method of Good *et al.* (10), and similarly corrected for non-fermentable reducing substances. Ketones were determined by the Ravin modification of Behre-Benedict method (11), and nitrogen by the macro-Kjeldahl method (12).

<div align="center">RESULTS</div>

Initial Response to Alloxan Injection. A triphasic glycemic response, namely, initial hyperglycemia, secondary hypoglycemia and then permanent diabetic hyperglycemia, was observed in all cases except *sheep 5* (table 1). Following the administration of alloxan, the blood glucose level rose steadily, reaching its peak of 92 to 217 mgm. per 100 ml. within 4 to 8 hours. A maximal hypoglycemia of 14 to 31 mgm. per 100 ml. was observed at the 9th to the 14th hours. Despite the low values of blood glucose, no symptoms of hypoglycemia were present. This phase was missed in *sheep 5*, in which hyperglycemia was present each time the blood glucose was determined, namely, at 4, 9, 11, and 13 hours postinjection.

Twenty-four hours after the injection, diabetic hyperglycemia had been established in all but one of the sheep. In *sheep 1*, which showed a prolonged hypoglycemia, blood glucose did not ascend to diabetic levels until the 38th hour.

Toxic effects of alloxan were observed in two animals. *Sheep 1* became weak 13 hours after receiving 125 mgm. of alloxan per kgm. Anuria was present from the 1th hour, and despite the administration of large amounts of saline and diuretics, 8t continued until death at 85 hours. As a consequence of renal failure, retention of iglucose, nitrogen, and ketones was severe; 72 hours after the injection of alloxan, the blood glucose level was 665 mgm. per 100 ml., blood nitrogen 167 mgm. per 100 ml. and blood ketones 36 mgm. per 100 ml. Histological studies showed both hepatic and renal tubular damage.

In *sheep 5*, which received 75 mgm. of alloxan per kgm., renal failure did not become apparent immediately; the blood non-protein nitrogen had been normal on the 8th day. Two days later, the blood glucose level rose from 233 to 341 mgm. per 100 ml., although the animal was fasting. On the 11th day, the hyperglycemia had reached 852 mgm. per 100 ml. Administration of 180 units of crystalline zinc insulin (Lilly) in the next 24 hours lowered the blood glucose to 75 mgm. per 100 ml. How-

ever, renal damage, evidenced by anuria present from the 10th day, caused death 12 days after alloxan administration.   Autopsy and histological studies showed glomerular and tubular damage in the kidney and fatty changes in the liver.

Alloxan Diabetic State.   a) Metabolic studies.   It can be seen from table 2 that the blood glucose levels of diabetic sheep are three to four times higher than those of normal sheep, in which the range is 35 to 65 mgm. per 100 ml.   That the

TABLE 1. INITIAL GLYCEMIC RESPONSE TO ALLOXAN INJECTION, MGM. PER CENT

HOURS POST-INJECTION	SHEEP NO.							
	1	2	3	4	5	6	7	8
0	45	56	38	40	54	63	40	56
½	79		56	35				
1	88	118	56	45				
2	118	167	68	56		154	58	84
3	143	191	84·	79				
4	165	200	91	107	165	182	114	217
5	167	189	92	121				
6	121	169				165	140	173
7	77	140	42	99				
8	26					140	134	112
9	22	77	14	54	184			
11	14		22	29	160	45	72	25
12	24	26						
13	22		19	26	165	31		
14	19						26	22
15		33	26	35		47		
16	19							
18		70						
19	17							
21		129						
22	24							
24		167	169	191	189	189	101	204
25	35							
27	47	184	193	209			158	255
30	38	191						
31			176					
33	68	211						211
38	213							

hyperglycemia is in large part alimentary is apparent from the comparison of values before and after fasting periods (table 3).   In two cases, *sheep 3* and *7*, normal values of blood glucose, urine glucose, and urine ketones were obtained after fasts of 6 and 5 days, respectively.   When the fast was prolonged in *sheep 3*, the blood glucose rose again to hyperglycemic levels.

In *sheep 6*, the first series of insulin treatments (protamine zinc insulin, Lilly) led to an increase in blood glucose, from 187 to 281 mg. per 100 ml., as a result of a great increase in food intake when on insulin therapy; this 5 months old ewe, with a body weight of 17 kgm., consumed approximately twice the adult intake of hay,

grain and commercial calf food in this period. Although the insulin dosage, 16 to 20 units daily, was inadequate at this time, the same dose proved to be too high at a later date when food intake was lower.

TABLE 2. VALUES OF BLOOD GLUCOSE AND KETONES, URINE GLUCOSE, KETONES, AND NITROGEN IN THE FED ALLOXAN DIABETIC SHEEP; AVERAGE VALUES AND RANGE

SHEEP NO.	POST-INJECTION	INITIAL WEIGHT	BLOOD GLUCOSE	BLOOD KETONES	URINE GLUCOSE[1]	URINE KETONES[1]	URINE NITROGEN
	days	*kgm.*	*mgm. %*	*mgm. %*	*g/kgm/day*	*mgm/kgm/day*	*mgm/kgm/day*
2	1–9	26.4	218 (202–246)	14	3.62 (0.31–5.61)		
3	1–11	47.3	171 (162–191)	24	2.39 (0.001–4.07)	131 (1.1–343)	507 (215–672)
4	1–10	36.8	175 (156–191)	47	1.64 (0.12–3.29)	129 (5.2–259)	418 (364–512)
5	1–3	44.1	187 (178–195)	51	1.75 (1.41–1.94)	160 (8.1–303)	400 (364–437)
6	1–12	17.0	173 (154–195)	41	1.98 (0.30–2.71)	238 (28.4–480)	415 (316–535)
7	1–9	17.7	148 (140–158)	11	1.00 (0.03–1.45)	4.1 (1.0–10.6)	362 (310–416)
7	10–20		146 (141–156)		1.38 (1.14–1.77)	24.5 (15.4–38.1)	341 (313–361)
8	1–7	27.7	190 (169–222)	12	1.65 (0.15–2.13)	102 (6.1–166)	316 (88–495)

[1] Low values on *day 1*.

TABLE 3. EFFECT OF FASTING IN ALLOXAN DIABETIC SHEEP

SHEEP NO.	FASTED	BLOOD GLUCOSE		BLOOD KETONES		URINE GLUCOSE		URINE KETONES		URINE NITROGEN	
		initial	termi-nal	initial	termi-nal	initial	terminal	initial	termi-nal	initial	termi-nal
	days	*mgm. %*		*mgm. %*		*g/kgm/day*		*mgm/kgm/day*		*mgm/kgm/day*	
2	6	206	173			2.79	0.15				
3	6	165	54			2.51	0.03	146	1.4	467	29
3	5	173	103			1.50	0.01	35	22	203	—
4	5	154	136			1.32	0.21	134	22	401	174
6	8	167	147			1.79	0.11	140	42	430	300
6	4	281	178	41	42	2.71	1.07	75	42	476	—
7	5	156	33			1.60	0.002	20	4	354	104
8	5	222	167	12	21	2.13	0.67	160	73	345	259

Glycosuria was severe in all sheep. The highest values were observed in *sheep 2*, which excreted over 5 grams of glucose per kgm. per day on the 2nd, 3rd, and 4th days after alloxan administration. Her blood glucose level never fell below 200 mgm. per 100 ml. during the period of study, except when the animal was fasting or receiving insulin. Glucose excretion was lowest in *sheep 7*, in which the blood glucose never rose above 158 mgm. per 100 ml. Nevertheless, it is believed that alloxan

damage to the pancreatic islets was complete, because a second injection of alloxan, on the 9th day, did not produce the typical initial glycemic fluctuations nor did it increase the degree of permanent diabetic hyperglycemia.

Fasting caused a rapid diminution of glycosuria in each of the alloxan diabetic animals. Normal values were obtained in all but 2 cases, the second fast of *sheep 6* and the fast of *sheep 8*. A marked drop in glucose excretion noted in the other sheep permitted the determination of the renal threshold at 140, 150, 150, 170, and 200 mgm. per 100 ml. of blood, in *sheep 4, 3, 7, 6,* and *2,* respectively. When the blood glucose fell below these levels, less than 0.5 grams of glucose per kgm. per day was excreted in the urine. No correlation was apparent between the renal threshold and the ability of these animals to maintain the blood glucose level on fasting.

Glucose tolerance curves were studied in both normal and diabetic sheep, following the intravenous injection of 1 gram of glucose per kgm. body weight (fig. 1). Compared with the dog and calf, the normal non-fasting sheep had a low glucose tolerance, similar to that obtained in an alloxan diabetic dog studied in this laboratory. The blood glucose level of the sheep did not return to the preinjection level until 4 to 6 hours after glucose administration, as compared with 1 to 2 hours in the normal dog and calf. Diabetes did not change the slope of the tolerance curve in sheep; the return to the preinjection level occurred 5 to 6 hours postinjection in most instances, although occasionally blood glucose remained slightly elevated throughout the remainder of the day. It must be remembered, however, that the initial level of blood glucose in the diabetic animal was three to four times higher than normal.

It is apparent from figure 2 that fasting caused a marked decrease in glucose tolerance in both normal and diabetic sheep. The high postinjection blood glucose levels did not return to normal during the course of the day in these animals.

Nitrogen excretion was increased in the diabetic animals. Control studies established the average normal non-fasting value at 220 mgm. of ammonia nitrogen per kgm. per day. After the administration of alloxan this rose to 396 mg. per kgm. per day. Although this approximately twofold increase is significant, it is much less than that reported in depancreatized dogs. Fasting decreased the nitrogen excretion in all sheep, but to the greatest extent in *sheep 3* and *7,* both of which failed to maintain hyperglycemia under these conditions. If urinary nitrogen excretion can be used as an index of the degree of gluconeogenesis from protein in the sheep, it seems doubtful that protein is of great importance as a source of carbohydrate, in these 2 animals in particular.

Disturbances in fat metabolism were observed in all animals which survived the first 24 hours. Accompanying the loss of body weight, ketonemia rose in these sheep from the normal level of 3 to 5 mgm. per 100 ml., to 11 to 51 mgm. per 100 ml. Ketosis was slow to develop in *sheep 7* and never became severe. The blood ketone level had decreased from 24 to 6 mgm. per 100 ml. in *sheep 3* by the 45th day, from 41 to 16 mgm. per 100 ml. in *sheep 6* by the 78th day, and from 11 to 9 mgm. per 100 ml. in *sheep 7* by the 94th day of alloxan diabetes. Blood ketone determinations were limited in number for any given animal because of the large blood sample required.

Ketonuria increased steadily from the normal value of 1 to 5 mgm. per kgm. per day in *sheep 3, 4, 5, 6,* and *8,* reaching a maximum of 166 to 480 mgm. per kgm. per day about a week after the production of diabetes. *Sheep 3* excreted a total of 16 grams of ketone bodies on the 8th day. Although the excretion values dropped after the peak had been reached, normal levels were not attained in these animals, except in *sheep 3* and *7* under conditions of fasting. Although the excretion of ketones was greatly decreased in the absence of a dietary source, this was apparently a renal phenomenon; the blood ketone level remained elevated in *sheep 6* and was doubled in *sheep 8* after fasts of 7 and 5 days, respectively. This suggests that the renal threshold for ketone bodies may be raised during fasting.

  b) General condition and survival (table 4). Of the 8 sheep studied, 5 animals presented a metabolic picture of uncomplicated alloxan diabetes. The data for *sheep 1* and *5,* with renal impairment, and *sheep 4,* which was in poor condition when
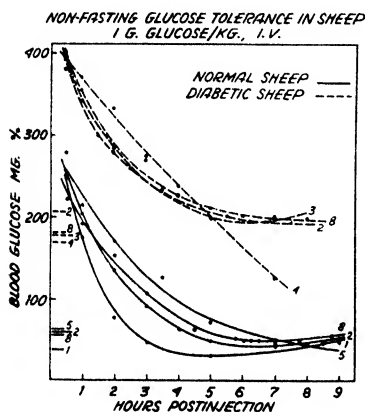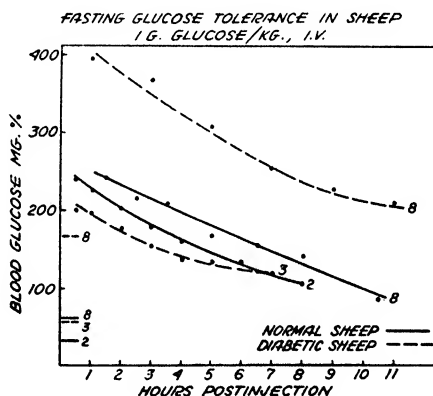
FIG. 1   FIG. 2

the experiment was started, are for these reasons excluded from this section. The general condition of the other sheep remained good for 1 to 5 months, despite the initial weight loss.

  In the first 2 weeks of alloxan diabetes, *sheep 2, 3, 6,* and *7* lost 17, 10, 11, and 15 per cent, respectively, of their body weights. At this time polyphagia became pronounced, and increased food intake compensated to some extent for the loss of glucose through the kidneys. Weight loss was excessive during periods of fasting. This is due, in part at least, to loss of rumen content.

  Sheep 2 was in good condition for about 45 days, at which time it developed weakness and failed rapidly. Insulin therapy was begun on the 50th day; 40 to 48 units of protamine zinc insulin (Lilly) were administered daily. The animal went into severe hypoglycemic coma on the 60th day, with a blood glucose level of 8 mgm. per 100 ml. Although glucose was administered intravenously several times during the next 24 hours, the condition did not improve; the animal was killed on the 61st day. The terminal glycemia was 10 mgm. per 100 ml.

  Sheep 3 remained in excellent condition for 150 days, maintaining body weight

by increased food intake during this period. An unfortunate mishap, not connected with the diabetes, resulted in death on the 157th day.

An 8-day fast in *sheep 6* caused a weight loss of 32 per cent, based on the initial weight. Insulin therapy was started on the 30th day of diabetes. Although 16 to 20 units of protamine zinc insulin (Lilly) were injected daily into this 17 kgm. lamb, the blood glucose level rose as a result of a great increase in food intake. Nevertheless, the animal regained weight and strength, and insulin was discontinued 16 days after its initiation. Progressive weakness was observed again from the 66th to the 71st day of diabetes, at which time insulin therapy was resumed. Two days later the lamb was comatose, shaking and salivating excessively. The blood glucose level was 22 mgm. per 100 ml. Intravenous administration of glucose brought the animal back to consciousness, but the weakness continued. The sheep was killed 78 days after the injection of alloxan, 6 days after the last insulin injection. The terminal blood glucose and ketones were 195 mgm. and 16 mgm. per 100 ml., re-

TABLE 4. SURVIVAL OF ALLOXAN DIABETIC SHEEP

SHEEP NO.	BREED	SURVIVAL	INSULIN THERAPY	CAUSE OF DEATH
1	Hampshire	85 hr.	None	Renal and hepatic damage
2	Hampshire	60 d.	days 50–58: 40–48 U protamine	Insulin hypoglycemia, following severe diabetic symptoms
3	Dorset	157 d.	None	Accidental
4	Hampshire	24 d.	None	Poor appetite and condition at start of experiment
5	Hampshire	12 d.	day 11: 120 U crystalline	Renal damage
6	Hampshire	78 d.	days 30–46, 71, 72: 16–20 U protamine	Severe effects of diabetes; killed
7	Shropshire	94 d.	none	Progressive weakness; killed
8	Hampshire	29 d.	day 21: 20 U protamine	Progressive weakness; killed

spectively. Although muscle glycogen was low normal, 0.38 per cent, liver glycogen was almost nonexistent, 0.04 per cent.

Sheep 7 was killed on the 94th day of alloxan diabetes. The animal's condition had remained good for over 2 months, but failed in the last few weeks and the sheep stood only when feeding during the last 2 or 3 days. Although the weight loss in 3 months of alloxan diabetes appeared to be slight, 1.3 kgm., it should be remembered that *sheep 7* was a growing lamb which should have been gaining weight in this period. The terminal blood glucose was 235 mgm. per 100 ml., ketones 9 mgm. per 100 ml. In this animal, muscle glycogen was normal, 0.43 per cent, but the liver was free of glycogen.

Sheep 8 showed progressive weakness from the 18th day, and was killed on the 29th day, 8 days after a single dose of 20 units of protamine zinc insulin (Lilly). The terminal blood glucose was 96 mgm. per 100 ml., terminal ketonemia 21 mgm. per 100 ml.; muscle glycogen was 0.46 per cent, liver glycogen 0.08 per cent. In one month of alloxan diabetes, this animal had lost 27 per cent of its body weight.

c) Histological studies (table 5). The renal damage which followed the injection of 125 and 75 mgm. of alloxan per kgm. in *sheep 1* and *5*, respectively, has been mentioned earlier. A large amount of ascitic fluid was removed from the peritoneal cavity of the former at autopsy, and other signs of renal failure were observed. Histological examination confirmed the diagnosis of coagulation necrosis of the convoluted tubules; many tubular casts were present. In *sheep 5*, the toxic effects of alloxan were apparent in both tubules and glomeruli; the latter were nonfunctional, compact masses of cells. *Sheep 3*, which died as a result of an accident, showed acute renal changes which were believed to result from the accident rather than from the diabetes. Renal pathology observed in three other animals consisted .

TABLE 5. HISTOLOGICAL STUDIES OF ALLOXAN DIABETIC SHEEP

SHEEP NO.	RENAL HISTOLOGY	HEPATIC HISTOLOGY	PANCREATIC HISTOLOGY
1	Tubular necrosis	Infiltration and proliferation of duct cells	No evident pathology
2	No evident pathology	Vacuolation of cells	Decreased number of islets
3	Acute hemorrhagic areas; tubular necrosis	Poorly preserved	Poorly preserved
4	No evident pathology	Vacuolation of cells; prominent bile ducts	Decreased number of islets
5	Vacuolation of tubular cells; massive proliferation of glomerular cells, glomeruli compact	Vacuolation of cells; prominent bile ducts	Decreased number of islets
6	Proliferation of glomerular cells	Connective tissue stimulation in region of portal vessels; some hepatic cells clear	No islets identified
7	Proliferation of glomerular cells	Vacuolation of cells; connective tissue stimulation in perivascular regions	One islet identified in section; possible remnants of other islets
8	Vacuolation of tubular cells	Vacuolation of cells; connective tissue stimulation in perivascular regions	Decreased number of islets; islets small

of proliferation of cells of the glomeruli in two and vacuolation of the tubular cells in one.

From table 5, it is apparent that hepatic changes were marked in all animals. In *sheep 1*, these were probably the result of the toxicity of alloxan. In those sheep which survived for longer periods, fatty infiltration of the liver was evident.

No islets could be identified in the pancreatic tissue of *sheep 6;* in the other animals there appeared to be a decrease in the number present. No pathological alterations were apparent in these with the routine hematoxylin and eosin staining technique. The islet cell types could not be differentiated in normal sheep pancreas even when stained according to the Gomori chrome hematoxylin technique (13). Pancreatic islet cells in the sheep are mainly nongranular, and the alpha and beta cells are stained very faintly (14).

DISCUSSION

The triphasic glycemic response to alloxan administration, observed in the sheep of this series, is typical of that presented by most other species studied. Although agreement is lacking in the explanations offered for the initial hyperglycemia and the secondary hypoglycemia, (15–18), all experimental results point to one cause for the third phase, the diabetic hyperglycemia. Assays of pancreatic tissue from alloxan diabetic animals have shown this to be the result of insulin deficiency, which is produced by the destructive action of alloxan on the beta cells of the pancreatic islets (15, 19). Studies of the alloxan diabetic state, for this reason, should provide a key to the action or actions of insulin in the normal animal.

Jarrett (7) observed severe but uncomplicated diabetes in 2 of 12 ewes treated with 88 to 200 mgm. of alloxan per kgm. One animal failed to develop diabetes; 2 were killed in the first 24 hours for histological studies, and 7 had serious hepatic and renal complications. Hyperglycemia, glycosuria and ketonuria were marked in both of the survivors, and large doses of insulin were required for control of the diabetes. The protocols of these animals are similar to those of our series.

In our sheep, intravenous administration of 75 mgm. of alloxan per kgm. resulted in uncomplicated diabetes in five cases, and in diabetes with renal involvement in one, *sheep 5*. In this instance, the fault appeared to be in the alloxan preparation, since it proved fatal to each of 5 rats to which it was administered in the usual manner and quantity.

Sheep 1, which received 125 mgm. of alloxan per kgm., died in 85 hours with coagulation necrosis of the renal convoluted tubules. No abnormality traceable to the toxic action of alloxan itself was found in the histological study of hepatic and renal tissue of *sheep 2*, 2 months after the injection of 100 mgm. of alloxan per kgm. It is apparent that 75 mgm. of alloxan per kgm. is the diabetogenic dose for sheep, and that doses greater than this may result in severe damage of the liver and kidneys.

After the initial period of readjustment to the metabolic abnormality, the glucose balance of the diabetic animals remains relatively constant, although this constancy is achieved through a turnover process in which the materials themselves are always changing. It is interesting to study the amount of carbohydrate present in the diabetic sheep in comparison with that in the normal. Muscle glycogen did not change significantly after the development of the diabetic state. The increase in blood glucose, and therefore an equivalent increase in tissue fluid glucose, is large in degree (122 mgm. per 100 ml.) but small in quantity (about 0.4 gram per kgm. body wt.). This increase does not balance the loss of liver glycogen (1.2 grams per kgm. body wt.), probably the most significant change in the glucose stores of diabetic sheep. From these results, it is apparent that the mechanism for net storage of glycogen in the liver is deficient, or that glucose mobilization from this organ is excessive even when the concentration of blood glucose without the aid of insulin maintains the muscle glycogen stores at approximately normal levels.

Despite the absence of liver glycogen, the total carbohydrate turnover of the diabetic sheep is higher than that of the normal sheep, when the amount of glucose excreted in the urine is taken into account. The source of the extra glucose in the

blood and urine of the diabetic carnivore is generally conceded to be the result of two processes: *a*) overproduction of glucose through hepatic gluconeogenesis and *b*) relative underutilization of glucose by the extrahepatic tissues.

Gluconeogenesis from body protein appears to be secondary as a source of blood glucose in diabetic sheep. The urinary nitrogen excretion was not quite doubled in these animals, and was less during fasting states, indicating a low degree of protein catabolism in both instances. The fed diabetic sheep excreted nitrogen equivalent to 2.4 grams of protein per kgm. per day, as compared to 1.5 grams per kgm. per day in the fed normal sheep. The fasted diabetic sheep excreted nitrogen equivalent to 0.2 to 1.8 grams of protein per kgm. per day. Little more than a gram of glucose per kgm. per day could arise, therefore, from protein catabolism in either fed or fasted conditions.

According to Barcroft *et al.* (20), the lower fatty acids arising from bacterial action on cellulose in the rumen are of great importance in the energy balance of ruminants. When absorbed, these may assume a major rôle in carbohydrate synthesis in this group of animals. Lorber and co-workers (21) have reported the appearance of labeled isotopic carbon atoms in liver glycogen produced *in vivo* from acetic acid. Other literature on gluconeogenesis from fat has been reviewed by Soskin and Levine (22). The presence of carbohydrate-fermenting bacteria in the rumen makes it doubtful that much, if any, glucose is absorbed as such into the portal circulation. Under these conditions, exogenous glucose is eliminated as the main source of blood glucose; gluconeogenesis is necessary for the maintenance of the blood glucose level in normal, non-fasting ruminants.

The type of glucose tolerance curve obtained in normal non-fasting sheep is similar to that of diabetic carnivores. In the latter, the low tolerance is the result of unchecked gluconeogenesis from body protein. In the normal sheep it seems probable that gluconeogenesis from absorbed lower fatty acids is responsible.

The difference between the glucose tolerance of normal and diabetic sheep lies in the blood glucose level; apparently the diabetic sheep at its high blood glucose level has a tolerance for injected glucose which is comparable to the tolerance for injected glucose exhibited by the normal sheep at its characteristic blood glucose level. This is essentially the same conclusion reached by Soskin and Levine (23) with respect to glucose utilization of diabetic dogs, namely, that the diabetic animal at its characteristically high blood glucose level utilizes as much glucose as the normal animal at its characteristic blood glucose level. A glycemia of 35 to 65 mgm. per 100 ml. is sufficient to drive an adequate carbohydrate metabolism in the normal sheep. A fourfold increase, as observed in the diabetics, would be expected to increase utilization four times in a normal sheep. Judging from the glucose tolerance, however, the glucose utilization of the diabetic sheep at this high level is similar to the normal sheep utilization at a level of 35 to 65 mgm. per 100 ml. At the same glycemic level, one would expect that the normal sheep utilizes four times as much glucose as the diabetic. A similar relationship exists between the normal and alloxan diabetic blood glucose levels of the dog, 90 to 100 mgm. per 100 ml. and 300 to 450 mgm. per 100 ml., respectively. If the glycemic level of the diabetic animal is adjusted so as to produce a normal rate of glucose utilization, one can con-

clude that the diabetic sheep exhibits the same degree of underutilization as does the diabetic dog.

A similar relationship exists between the glucose tolerance curves of fasting normal and fasting diabetic sheep, that is, the same form of curve with similar slope occurs at different blood glucose levels. A comparison of the tolerance under fed and fasting conditions, however, reveals a marked decrease in the latter. It is apparent that a condition of fasting causes physiological responses which are not evoked by the diabetic state in sheep.

It has already been mentioned that excessive gluconeogenesis from body protein is responsible for the low glucose tolerance of the fasting normal carnivore. An increased gluconeogenesis could be effected through a pathway described by Long (24), who has found that adrenaline secretion stimulates the anterior pituitary gland to secrete the adrenocorticotropic hormones which are believed to increase gluconeogenesis, in part at least, by mobilizing body proteins and fats. Since the output of adrenaline into the blood is known to be increased during hypoglycemic states, the latter is a probable factor in controlling the gluconeogenic process. In *sheep 2* and *3*, the glycemias before glucose administration were subnormal with respect to their characteristic levels; hypoglycemia was not so apparent in the fasting diabetic tolerance study on *sheep 8*, and was absent in the study carried out in this animal before the production of diabetes. It should be pointed out that hypoglycemic symptoms are known to occur at higher blood sugar levels in diabetic animals, in which glucose utilization is low. Although it is probable that the decreased glucose tolerance of the fasted sheep is the result of mobilization of fat and, to a lesser extent, body protein, the complete mechanism which causes this mobilization cannot be identified with certainty at this time.

In order to gain an idea of the relative importance of dietary factors in diabetes in sheep and dogs, it is necessary to study the fasting values in comparison to those obtained when the animals were on full feed. Because food intake was estimated rather than measured, and also because of the variation in digestibility and caloric value of the feed, it is impossible to express the alimentary factor quantitatively.

Wastage of glucose and ketone bodies through urinary excretion continues when diabetic carnivores are fasted. Both are diminished in fasting diabetic sheep, the former to the vanishing point. This marked drop in glucose excretion must be explained by a high renal threshold, which threshold is close to that of dogs. It is possible, also, that exogenous gluconeogenesis accounts for a large proportion of the glucose excreted when the animals are fed.

It is harder to explain the decreased ketonuria. Ketosis of fasting has been observed in both dogs and humans; ketosis also occurs in pregnancy disease of ewes, which may be the result of undernutrition. However, in the fasting diabetic sheep, ketonuria was low in degree, although ketonemia continued. Thus, diabetic ketonuria like glycosuria in sheep appears to be largely of dietary origin.

In 4 out of 6 sheep, the diabetic hyperglycemia was maintained under fasting conditions. This indicates an endogenous source of carbohydrate precursors. As has been mentioned previously, the lower fatty acids are important sources in the ruminant.

In *sheep 3* and *7*, Dorset and Shropshire ewes, respectively, normal blood glucose levels were obtained when the animals were fasted. Both of these animals seemed to suffer less from diabetes than the other animals, which were Hampshires. The former animal lived 5 months without benefit of exogenous insulin, and by excessive food intake, regained the weight lost in the initial period of alloxan diabetes. *Sheep 7* was sacrificed after 94 days of alloxan diabetes; insulin was not necessary for survival. Ketosis, as measured by the degree of ketonemia, was mild in both animals, and ketonuria decreased to normal when they were fasted; the fasting values of urine nitrogen were low. The resemblance to the Houssay preparation, the depancreatized-hypophysectomized dog, is striking. It should be pointed out that gluconeogenesis from body protein is greatly reduced after hypophysectomy; Braier (25) reported low levels of nitrogen excretion in fasting hypophysectomized dogs, similar to the levels obtained in the fasting diabetic sheep.

SUMMARY

Intravenous administration of alloxan monohydrate produced the typical triphasic response to alloxan injection, namely, initial hyperglycemia, secondary hypoglycemia and tertiary permanent diabetic hyperglycemia in 7 sheep which were so treated. Seventy-five mgm. of alloxan per kgm. was found to be the diabetogenic dose for sheep. Higher doses frequently cause severe renal and hepatic damage.

Severe hyperglycemia, glycosuria, ketonemia, and ketonuria developed in the 6 sheep which did not suffer renal damage. Urinary nitrogen excretion was increased almost twofold in these animals. The ketosis decreased after the first few weeks of diabetes.

Fasts of 4 to 8 days caused a diminution or disappearance of glycosuria. Ketonuria and urinary nitrogen excretion were also decreased. Ketonemia continued throughout the fast. The blood glucose level fell to normal in two instances, and in six others the reduction was of less degree.

Polyphagia caused an increase of 100 mgm. per 100 ml. of blood in the degree of hyperglycemia in one instance. The influence of absorbed lower fatty acids upon intermediary metabolism of normal and diabetic sheep is discussed. Body protein apparently is secondary as a carbohydrate source in the sheep; gluconeogenesis from dietary volatile fatty acids assumes a more important rôle in this regard. Underutilization of glucose is also prominent in diabetic sheep.

A weight loss of 10 to 17 per cent was observed in the first two weeks of alloxan diabetes. Increased food intake later compensated in part for this loss. Survival of those animals presenting cases of uncomplicated alloxan diabetes was relatively long, from 1 to 5 months, without continued insulin therapy.

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A COMPARISON OF GLUCOSE METABOLISM AFTER HEMOR-
RHAGE IN NON-DIABETIC, ALLOXAN DIABETIC AND
DEPANCREATIZED DOGS

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WHEN dogs are subjected to 75 per cent hemorrhage by Walcott's method (1), their arterial blood glucose concentration rises rapidly, reaching a maximum value at varying times after hemorrhage and almost invariably showing a sharp terminal decrease (2). Furthermore the animals also show an initial increase in the A-V glucose difference (peripheral glucose utilization), followed by a terminal decrease. However, glucose utilization does not necessarily rise and fall simultaneously with the rise and fall of the arterial blood glucose level. Moreover, the intravenous injection of sufficient glucose to raise the blood sugar level of normal dogs to approximately the height caused by a 75 per cent hemorrhage does not raise the A-V difference to the high value found after bleeding. This is true even when one takes into account the differences in blood flow (3). Changes in A-V glucose difference are dependent not only upon the arterial blood glucose level and the rate of blood flow, but also upon one or more additional factors. All or nearly all the rise in the arterial blood glucose concentration after hemorrhage is caused by the secretion of epinephrine (3, 4). However, epinephrine hyperglycemia has little influence on glucose utilization (5–7), although epinephrine is known to increase the muscle plasma glucose ratio (8). These findings indicate that some factor other than epinephrine secretion—changes in the rate of blood flow, or increased arterial blood glucose level—appears to increase the glucose A-V difference after hemorrhage. Since insulin is known to increase the rate of removal of blood glucose by peripheral tissues (6, 9) the following experiments were performed to determine whether or not changes in the secretion of insulin are concerned with variations in the glucose A-V difference (glucose utilization) induced by a 75 per cent hemorrhage.

METHOD

Animals in which the secretion of insulin was decreased or abolished were prepared by the administration of alloxan or by pancreatectomy. The use of alloxan injection rests upon the evidence that this substance specifically destroys the beta cells of the pancreas (10–13).

Seventeen dogs, weighing 7 to 13 kg., were injected intravenously with 50 to 60 mg/kg. of alloxan monohydrate dissolved in 10 cc. of distilled water. These animals were fasted for 18 to 24 hours before injection. The dogs were kept for 3 to 11 days after the injection of alloxan, blood glucose determinations being made every other day. If the blood glucose concentration did not increase above the control value or rose and returned to the preinjection level, additional injections of

50 to 150 mg/kg. of alloxan were given until the arterial whole blood glucoes concentration stayed above 200 mg. per cent.   When the animal appeared to be in a permanent state of hypoinsulinism, it was fasted for 18 to 24 hours and subjected to a 75 per cent hemorrhage by Walcott's technique (bled out and 25 per cent of the bleeding volume immediately returned).

Total pancreatectomies were made on 9 dogs weighing between 8 and 13 kg.   During the four to seven days following the operation, each animal was given 3 to 10 U of protamine zinc insulin[2] per day.   This maintained the blood glucose concentration at the pre-operative level.   When the wound had healed and the animal was in good condition, insulin was discontinued and two to five days later the dog was fasted 18 to 24 hours and hemorrhaged 75 per cent of its bleeding volume.

Glucose, lactate and pyruvate analyses were made on whole blood. $ZnSO_4$ and $NaOH$ were used to precipitate the blood proteins for the glucose and lactate analyses.   Glucose determinations were made according to Somogyi's method (14) and lactate and pyruvate analyses were carried out as previously described (2).   Plasma bromsulphaleins were determined in a Coleman Junior Spectrophotometer, the color being developed according to the method given in Peters and Van Slyke (15). The thymol flocculation test was carried out on heparinized plasma as described by Maclagan (16). The liver was examined histologically for fat by staining frozen sections with Sudan IV, the tissues being previously fixed in 10 per cent formaldehyde.   Plasma volume determinations were done with the blue dye, T-1824 (17).   Hematocrit readings were made on heparinized blood by centrifuging for 30 minutes at 3000 r.p.m. in Wintrobe tubes.

The relative reduction in the volume of blood flowing through the hind limb following a 75 per cent hemorrhage was estimated by inserting a T-shaped cannula into the femoral vein of dogs in which the blood had been heparinized using the dosages recommended by Solandt and Best (18). After clamping the femoral vein the blood from the side arm of the cannula was collected for a measured time interval.   It is recognized that this procedure measures only the relative variations in the blood flow.

<div align="center">RESULTS</div>

The injection of alloxan has been reported (12) to cause liver damage.   Since the extent to which such liver damage might influence glucose metabolism is unknown, the amount of injury in the livers of the first few dogs injected with alloxan was investigated.   All of the thymol flocculation tests done on 7 dogs before and after the injection of alloxan were negative (table 1), with the exception of *dog 10*, in which the test became 1+ when the animal developed distemper.   Seven dogs, some of which were used for the thymol flocculation test, were injected with 4 mg/kg. of bromsulphalein.   The administration of alloxan produced no change in the disappearance rate of bromsulphalein (table 1).   *Dog 10*, which contracted distemper and showed a 1+ thymol flocculation test, also showed a significantly higher 60-minute plasma bromsulphalein level.   The livers from 9 alloxan diabetic dogs were stained with Sudan IV and examined for fat (table 1).   In 5 of these animals the fat content of the liver increased.   The other 4 animals had no more fat in their liver than is often present in uninjected control animals; only an occasional parenchymal cell or cluster of cells containing fat was seen.   Fat was, of course, found in the bile duct epithelium of both control and injected animals.   When marked fatty infiltration occurred the fat was distributed in the area surrounding the central veins. Further liver function tests were not done because the data presented later in this paper gave no indication of any impairment in the ability of the liver to produce glucose after the administration of alloxan even in the presence of considerable fatty infiltration.

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[2] Eli Lilly and Co. Indianapolis, Ind.

The utilization of glucose before and after a 75 per cent hemorrhage was studied on 13 alloxan diabetic and 7 depancreatized dogs. A decrease in blood glucose as the blood passed through the tissues of the hind limb (negative A-V difference) was arbitrarily termed glucose utilization. The disappearance of glucose from the blood stream may not signify tissue utilization, but as is shown later the A-V difference may be used as an index of glucose utilization under the conditions of these experiments.

TABLE I.　DOSE OF ALLOXAN GIVEN TO THE DOGS AND THE CHANGE IN THE BLOOD GLUCOSE LEVEL AFTER THE INJECTION OF THE ALLOXAN, COMPARED WITH THE AMOUNT OF LIVER DAMAGE

DOG	WHOLE BLOOD GLUCOSE IN MG. %			DOSE OF ALLOXAN	DAYS AFTER ALLOXAN BEFORE TERMINUS	LIVER FAT CONTENT	THYMOL FLOC. TEST[1] AFTER ALLOXAN[2]	BROMOSULPHALEIN LEVELS IN PLASMA AFTER 4 MG/KG. OF DYE			
	Before alloxan, control	After alloxan						Before alloxan[9] (mg. %)		After alloxan[9] (mg. %)	
		Max.	Terminal					10 min.	1 hr.	10 min.	1 hr.
				mg/kg.							
1	53	271	101	53[3]	8	+					
2	61	298	273	50[3]	37	+	neg. 8 days[6]				
							neg. 25 days				
3	73		[4]	50	1	++++					
4	73	206	111[5]	50[7]	11	++	neg. 4 days[8]				
				75			neg. 6 days				
5	60		533[5]	50[7]	3	+		.3	.1		
6	65	153	85	50[3]	7	+	neg. 2 days	.3	o	.2	o
							neg. 4 days			.5	o
7	71		787[5]	60[7]	4	+++					
8	65		359[5]	55[7]	5	+++	neg. 2 days	.5	.1		.2
							neg. 3 days				
9	55	298	136[5]	52[7]	5	++	neg. 2 days	.6	.2	1.2	.4
10	55	573		55[3]	6		neg. 3 days			.7	.2
							1 + 6 days	.5	.2	1.3	.8
11	73	220	197	50	20		neg. 14 days	.3	.1	.4	.1
12[10]		82		50				.8	.1		

[1] All thymol flocculation tests before alloxan were negative.　[2] Blood glucose above 200 mg.%.　[3] Experiments terminated with nembutal.　[4] Died hypoglycemic convulsion.　[5] Control blood sugar on day of experiment (75% hemorrhage).　[6] Number of days after alloxan before test was performed.　[7] Liver studied following a fatal 75% hemorrhage.　[8] Blood glucose of 103 mg.%.　[9] Bromsulphalein excretion tests performed on same days as thymol flocculation tests.　[10] Dog did not develop diabetes.

Glucose A-V differences were determined by taking simultaneous (within less than 2 minutes) samples of blood from the femoral artery and vein. The individual and average arterial whole blood glucose concentrations and glucose A-V differences of non-diabetic, alloxan diabetic and pancreatectomized animals before hemorrhage are given in tables 2, 3, and 4. The arterial blood glucose levels before bleeding were higher in the diabetic than in the non-diabetic dogs. However, the pre-hemorrhagic A-V glucose differences of the three series of animals were the same.

Following hemorrhage the general pattern of glucose metabolism in the diabetic

animals was similar to that found in the non-diabetic dogs. The arterial blood glucose level rose to a maximum value. This maximum glucose concentration occurred at any time after bleeding except terminally (table 2). In the last 30 minutes of existence there was almost invariably a decrease in the glucose concentration of 10 to 110 mg. per cent. The average maximum increase after bleeding in the arterial blood

TABLE 2. RELATIONSHIP OF ARTERIAL GLUCOSE CONCENTRATION (IN MG. %) TO THE FEMORAL A-V GLUCOSE DIFFERENCE IN ALLOXAN DIABETIC AND DEPANCREATIZED DOGS BEFORE AND AFTER A 75% HEMORRHAGE

DOG	CONTROL		TIME AFTER A 75% HEMORRHAGE										SURVIVAL AFTER HEMORRHAGE	DOSE OF ALLOXAN	VENOUS GLUCOSE LEVEL BEFORE ALLOXAN
	Art.	A-V	1 hr Art.	1 hr A-V	2 hr Art.	2 hr A-V	3 hr Art.	3 hr A-V	4 hr Art.	4 hr A-V	5 hr Art.	5 hr A-V	hr.	mg/kg.	
Alloxan Diabetic															
5	533	—7	668	—43	688	—8	698	—5	516				4	50	60
7	787	+6	881	—96	885	—44[1]							1.3	60	71
8	359	—8	460	—51	401	—3							2	55	65
13	248	—8	413	—68	416	—60	395	—48	287				4.3	55, 55	90
14	247	—0	311	—25	440	—44	535	—65	551	—43			4.3	52	86
15	273[2]	—5	423	—47	394	—25							2	53	76
16	286[2]	—3	670	—132	744	—172	790	—134	790[3]	—94			3.5	53	80
17	223	—6	400	—62	419	—47	478	—50	508	—50	575	—61	5.5	55, 55	
18	205	—1	405	—26	447	—27	498		495		582 / 557[4]		5.8	55, 80, 100, 100	72
19	252	—2	349	—30	394	—35	405	—20	442	—37	377[5]	+13	6	55	83
20	233	—8												57	67
21	412	—4	610	—20	662	—16	686	—10	714	—12	640	+15	5	57	60
22	278[2]	0	593	—34	575	—44	524	+2					4	55, 60, 65, 75, 100, 100, 100, 150, 150	88
23	349[2]	—2	468	—40	520	—56	543	—15	501	—21			5	55	
24	312[2]	0	441	—16	459	—12	492	—21	485	0			4.3	55, 55	111
Depancreatized															
1	254	—6	295	—33	336	—58	340	—52	274[3]	+1			3.5		
2	303	—13	379	—30	430	—35	388	—0					2.5		
3	385	—21	555	—83	624[6]	—84	608[7]	—68	590[8]	—57			2.5		
4	244	—12	330	—52	378	—75	412	—66	372	—41			4		
5	303[2]	0	432	—49	399	—13	338	+4					3		
6	299[2]	—8											2.5		
7	358[2]	—3	478	—27	512	—54	450						3		
8	285	+2	405	—75	489	—77	568	—81	600	—69	552[9]	—39	4.5		

[1] This was a 1.3-hour sample. [2] This animal heparinized. [3] This was a 3.5-hour sample. [4] This was a 5.8-hour sample. [5] This was a 6-hour sample. [6] This was a 1.5-hour sample. [7] This was a 2-hour sample. [8] This was a 2.5-hour sample. [9] 4.5-hour sample.

glucose concentrations of the alloxan diabetic animals was 109 mg. per cent greater than that of the control animals (table 3) while this value was only 52 mg. per cent for the dogs of the pancreatectomy series. Since differences in the concentrations of glucose could be explained by differences in circulatory volume produced by alloxan injection, plasma volume determinations were carried out on 6 alloxan diabetic dogs both before the injection of alloxan and either on the day preceding or on the day of the bleeding experiment. It was found that any changes in the plasma or blood

volume (calculated from dye and hematocrit readings) of the dogs after the injection of diabetogenic doses of alloxan were within the error of the method. After the administration of alloxan the plasma volume increased an average of 1.5 per cent (+8 to −9 per cent) and the blood volume decreased an average of 5.0 per cent (+4 to −11 per cent). As in the control dogs, the post-hemorrhagic femoral A-V glucose difference in the diabetic animals increased to a maximum after which a progressive

TABLE 3. WHOLE BLOOD GLUCOSE (ARTERIAL) CONCENTRATIONS AND FEMORAL A-V DIFFERENCES (MEANS) BEFORE AND AFTER 75% HEMORRHAGE

All values expressed in mg/100 cc., as mean and standard error $\frac{o}{(\sqrt{n})}$ of the mean, n = no. of animals

	CONTROL SERIES, C[1]	n	ALLOXAN SERIES, A	n	DEPAN. SERIES, D	n	DIFF. IN MEANS / S.E. DIFF.
Before hemorrhage							
Blood glucose	77 ± 1.7	25	330 ±35	17	304 ±17	8	
A-V diff.	3.2 ± 0.5	25	3.2 ± 1.2	17	7.6 ± 2.7	8	C & A, no diff. C & D, 1.6 A & D, 1.6
After hemorrhage							
Max. rise above pre-hemorrhage level of blood glucose	122 ±20	12	231 ±32	13	174 ±30	7	C & A, 2.9 C & D, 1.5 A & D, 1.3
Max. rise above pre-hemorrhage level of A-V diff.	33 ± 3.2	12	57 ±11	13	55 ± 7	7	C & A, 2.1 C & D, 2.9 A & D, 0.2

[1] Values taken from paper of Beatty (2).

TABLE 4. AVERAGE PERCENTAGE REDUCTION IN BLOOD FLOW AFTER 75% HEMORRHAGE

	1 HOUR	2 HOURS	3 HOURS	TERMINAL[1]
Control (8 dogs)	24[2]	27	21[3]	20
Alloxan diabetic (7 dogs)	22	21	18[3]	16[2]
Pancreatectomized (3 dogs)	25	20	20	19

[1] Within the last ½ hour of survival.   [2] This average is for 6 dogs.   [3] This average is for 5 dogs.

decrease occurred until death supervened. However, the average maximum femoral A-V glucose difference rose to a greater extent in the two diabetic groups than in the control, non-diabetic series (table 3). In 2 dogs with alloxan diabetes and 2 pancreatectomized animals the terminal glucose A-V difference was postive. No positive terminal A-V glucose differences were observed in any of the 11 dogs of the control series. It is interesting to note that positive A-V glucose differences have been observed in intravenous glucose tolerance curves in normal animals (3, 19). Glucose A-V differences were not corrected for changes in the hematocrit reading as the blood flowed through the leg, for the diabetic A-V hematocrit differences were small,

amounting to 0 to 2.1 units, and were not changed by hemorrhage (six determinations). Similar results have been reported previously for non-diabetic dogs (2).

Differences in the glucose A-V differences produced in the three series of animals by bleeding could be caused by variations in blood flows. For this reason, the relative change in the blood flow through the femoral vein following a 75 per cent hemor-

Fig. 1. SHOWING THE SIMILARITY of the patterns of changes in arterial blood glucose concentration and glucose A-V differences following a 75 per cent hemorrhage in control, alloxan diabetic and pancreatectomized animals. Unbroken lines represent arterial blood glucose concentration and broken lines, glucose A-V differences.
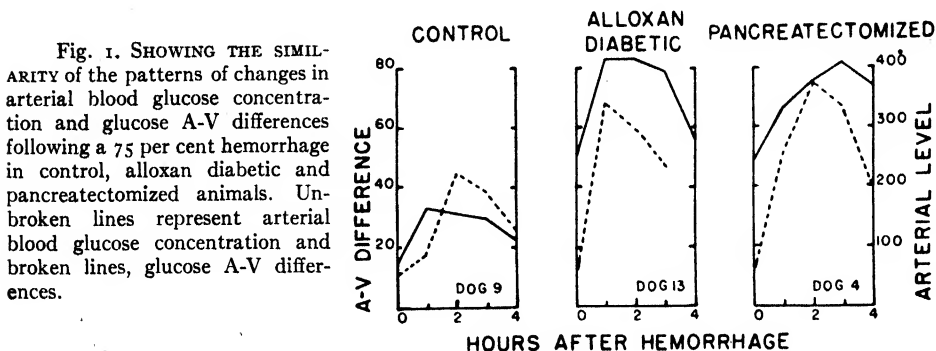
TABLE 5. COMPARISON OF WHOLE BLOOD LACTATE, PYRUVATE AND L/P RATIOS IN CONTROL, ALLOXAN DIABETIC AND PANCREATECTOMIZED DOGS, BEFORE AND AFTER A 75% HEMORRHAGE

All values expressed in mg/100 cc., as mean and standard error o/(vn) of the mean. n = no. of animals

SERIES	BEFORE HEMORRHAGE			1 HR. AFTER HEMORRHAGE			TERMINAL[1]		
	Lactate	n	Diff. of means / S.E. diff.	Lactate	n	Diff. of means / S.E. diff.	Lactate	n	Diff. of means / S.E. diff.
	mg. %			mg. %			mg. %		
Control, C	12 ±1.4	36		58 ± 3.0	36		109 ± 4.3	35	
Alloxan, A	12 ±1.5	6	C & A, no diff.	51 ± 6.7	7	C & A, 1.9	118 ± 6.0	7	C & A, 1.2
Depancreatized, D	27 ±2.4	3[2]	C & D[3]	63 ±10.7	5	C & D, 0.45	120 ±16.2	5	C & D, 0.7
	Pyruvate			Pyruvate			Pyruvate		
Control, C	1.4 ±0.14	31		4.6 ±.20	21		4.2 ±0.20	29	
Alloxan, A	1.7 ±0.19	11	C & A, 1.2	3.2 ±.35	10	C & A, 3.5	4.7 ± .27	11	C & A, 1.5
Depancreatized, D	1.7 ±0.04	5	C & D, 2.1	2.9 ±.24	5	C & D, 5.4	5.1 ± .44	5	C & D, 1.9
	L/P			L/P			L/P		
Control, C	8 ±2.3	31		12 ±0.7	21		29 ±1.6	27	
Diabetic, D	11 ±1.4	6[4]	C & D, 1.1	20 ±2.0	9[4]	C & D, 3.8	25 ±2.3	9[4]	C & D, 1.5

[1] Within the last 30 min. of survival.   [2] Too short a series to show significant difference.   [3] Combined series consisting of 4 alloxan diabetic and 2 pancreatectomized dogs.   [4] Combined series of 5 alloxan diabetic and 4 pancreatectomized dogs.

rhage was measured in all the series. In the animals of all three groups the limb blood flows decreased immediately after bleeding to an average of 22 to 25 per cent of the pre-hemorrhagic values (table 4). No further marked change in blood flow occurred unless determinations were made within the last 10 minutes of survival when further rapid decreases in flow were observed. When the blood flow through the femoral vein was calculated as cc/kg. of body weight, it was found that 10 control

dogs had an average flow of 4.2 cc/kg., 6 alloxan diabetics 4.4 cc/kg. and 3 depancreatized 4.3 cc/kg.   These results indicate that differences in the peripheral rate of disappearance of glucose between the control and the two diabetic series cannot be related to differences in the blood flow through the hind limb.   Crandall and Lipscomb (20) likewise found little change in the blood flow through the liver of dogs after pancreatectomy.

Before hemorrhage the average blood lactate and pyruvate values and L/P ratios in the control, alloxan diabetic and depancreatized dogs showed no differences, except for the increased lactate concentration of the depancreatized animals (table 5). Since lactate and pyruvate determinations were not always made on the same animals, the L/P ratios of the two diabetic groups were combined in order to obtain a number large enough for comparison with those of the control group.   The rise in blood lactate concentrations after hemorrhage (table 5) was similar in the three groups of animals.   The blood pyruvate levels also rose (table 5), but the concentrations the first hour after bleeding were lower in the alloxan diabetic and depancreatized series than in the control series.   At this time the average L/P ratio of the combined diabetic group was higher than that of the control series.   The average terminal blood pyruvate levels were not different in the three series of animals, nor was there any difference between the terminal L/P ratios of the combined diabetic and control groups.

Comparison of the bleeding volumes in cc/kg. shows that there were no differences between the values obtained on the control animals and those of the two diabetic series (table 6).   The bleeding volumes were calculated on the basis of the dog's weight on the day of the experiment.   The average survival time of the three series of animals was also similar, $3\frac{3}{4}$ hours for the control group, 4 hours for the alloxan diabetics and $3\frac{1}{4}$ hours for the depancreatized.

Since anemia has been reported in man after alloxan injection (21), hematocrit determinations were made on 10 dogs before and after the injection of varying doses of alloxan (table 7).   In 9 of 10 animals the hematocrit values decreased.   The total circulating red cell volume was calculated for 7 dogs in which plasma volume had been determined (table 7).   In 6 of 7 dogs a decrease of 3 to 20 per cent (average 14 per cent) in the total circulating red cell volume was found.

DISCUSSION

There were no significant differences in the average pre-hemorrhagic femoral A-V glucose differences (table 3), nor in the relative blood flows of the alloxan diabetic, depancreatized and control series.   These findings indicate, in agreement with Soskin and Levine (22), that, on the average, the blood glucose level in our diabetic animals was high enough to enable the mucles of the hind limbs to utilize sugar in approximately the same amount as in the control, non-diabetic animals.

Statistically speaking, the increase in the arterial blood glucose level of the hemorrhaged depancreatized dogs was equivalent to that of the control dogs, whereas the rise in glucose concentration in the animals of the alloxan diabetic series was greater than that in the control group (table 3).   A possible difference between the alloxan diabetic and the depancreatized dogs may be explained by the less favorable physical

condition of the depancreatized animal in comparison with the alloxan dog. Pancreatectomy caused a greater drop in weight than injection of alloxan, although the two series of dogs were diabetic for similar periods of time. However, the differences in weight loss between the two diabetic groups was not significant (table 6). Another explanation for a difference between the two diabetic series may be the presence of a small number of functioning beta cells in the alloxan diabetic dog. A third explana-

TABLE 6. COMPARISON OF BLEEDING VOLUMES AND SURVIVAL TIMES IN CONTROL, ALLOXAN DIABETIC AND DEPANCREATIZED DOGS UNDERGOING A HEMORRHAGE OF 75% OF THE BLEEDING VOLUME

All values expressed in mg/100 cc., as mean and standard error o/(vn) of the mean. n = no. of animals

SERIES	BLEEDING VOL.	n	DIFF. OF MEANS / S.E. DIFF.	WT. LOSS	n	DIFF. OF MEANS / S.E. DIFF.	SURVIVAL TIME	n	DIFF. OF MEANS / S.E. DIFF.
	cc/kg.			*kg.*			*hr.*		
Control, C	60 ±1	72		0.5 ±0.2[1]	21		3.75 ±0.3	14	
Alloxan, A	54 ±3	14	C & A, 1.8	0.9 ±0.1[2]	13	C & A, 1.6	4.00 ±0.4	15	C & A, 0.5
Depancreatized, D	56 ±3	8	C & D, 0.9	1.4 ±0.4	6	C & D, 2.2	3.25 ±0.3	8	C & D, 1.2

[1] Weight loss during the 18- to 24-hr. fast preceding the experiment.   [2] Weight loss during the entire diabetic period, including the 18- to 24-hr. fast preceding the experiment.

TABLE 7. HEMATOCRIT VALUES AND TOTAL CIRCULATING RED-CELL VOLUMES BEFORE AND AFTER THE INJECTION OF DIABETOGENIC DOSES OF ALLOXAN

DOG	HCT. BEFORE ALLOXAN	HCT. AFTER ALLOXAN	CHANGE IN HCT.	% CHANGE IN TOTAL RED-CELL VOL.[1]	DOSE OF ALLOXAN	DAYS BETWEEN 1ST ALLOXAN AND HCT. DETER.
					mg./kg.	
25	50.5	46.0	−4.5	−20	55, 55	6
18	34.0	26.0	−8.0	−10	55, 80, 100, 100	9
26	46.3	40.7	−5.6	−19	55, 60	5
20[2]	37.6	49.5	+11.9		57	6
21	47.7	41.1	−6.6	−3.0	57	3
22[3]	42.6	41.7	−0.9		3.3 gm.[4]	15
23	47.5	41.0	−6.5	−17	55	3
24	41.7	41.5	−0.2	+2	55, 55	3
27	38.0	32.0	−6.0	−14	55	3
19	38.9	35.7	−3.2		55	7

[1] No correction made for relatively constant errors of trapped plasma and buffy coat.   [2] Animal vomited frequently and did not eat well.   [3] Animal vomited 3 times immediately following injections of alloxan but ate well.   [4] Total alloxan over a period of 13 days.

tion might be that some pancreatic cells other than the beta cells are secreting a hypothetical second pancreatic hormone (23) that is present only in the alloxan diabetic dogs in which the beta cells are destroyed without permanent damage to other islet cells. Since the number of dogs in the depancreatized series was small for statistical purposes and the range of values large and since there was no significant difference between the post-hemorrhagic increments in arterial blood glucose levels of the two diabetic groups, all the diabetic dogs have been combined for comparison with the

control animals. When this is done a statistically significant difference can be demonstrated between the control and combined diabetic series (diff. in means/S.E. diff. = 2.9). The greater post-hemorrhagic rise in arterial blood glucose level in the alloxan diabetic dogs when compared with the control dogs cannot be explained on the basis of a decreased plasma or blood volume, because diabetogenic doses of alloxan produced no significant change in the plasma or blood volume. Therefore, the greater rise in blood glucose was probably caused either by the liver producing more glucose or by the tissues removing less glucose, or by a combination of both processes.

The average maximum femoral A-V glucose difference rose to a greater extent after hemorrhage in the two diabetic than in the non-diabetic series (table 3). It is somewhat doubtful whether the increment in the A-V difference of the alloxan diabetic dogs is statistically greater than that of the control dogs. However, the rise in the A-V difference of the depancreatized animals is clearly larger than that of the non-diabetic dogs. For the reasons stated above a comparison was made of the combined diabetic and control series and again a statistically significant difference between the two series was demonstrated (diff. in means/S.E. diff. = 3.8). The difference between the post-hemorrhagic A-V differences cannot be explained by differences in the blood flow through the hind limb because the relative changes in the blood flow through the hind leg after bleeding were similar in the control, alloxan diabetic and depancreatized dogs (table 4). A-V differences measure the disappearance of glucose from the blood stream and not necessarily utilization. The A-V difference in these experiments indicates that glucose is continually leaving the blood stream. Since equilibrium is rapidly attained, the glucose must be leaving the interstitial fluid compartment and entering the cells, in order to maintain the concentration in the interstitial fluid compartment close to the blood level. Storage of glucose as glycogen in the cells appears impossible in view of Tachi's finding (24) that severe hemorrhage causes a marked decrease in the muscle glycogen of non-diabetic rabbits. Furthermore, the rate of glycogen synthesis in the muscles, and especially synthesis from glucose, is decreased in animals which lack sufficient insulin (25, 26).

The stimulating effect on glucose utilization of the greater post-hemorrhagic increase in the blood glucose level of diabetic animals when compared with non-diabetic animals might explain the greater A-V difference in the alloxan diabetics. However, raising the arterial blood glucose concentration by intravenous injection of glucose in non-diabetic dogs increased the A-V difference less than increasing the arterial blood glucose level the same amount by hemorrhage (taking blood flow into account) (3). Intravenous glucose injections were used because ingestion by mouth raised the blood glucose level too slowly. Injection of 0.75 mg/kg. of glucose by vein increased the blood glucose concentration to about the same level as that reached by hemorrhage in a similar interval of time. As in the control series, the glucose A-V difference did not necessarily follow the arterial blood glucose concentration (for example see *dog 22*, table 2). Insufficient oxygen for utilization could be the direct cause of a decrease in the peripheral glucose utilization even though the arterial blood glucose level was rising, but an additional mechanism is necessary to explain how the A-V glucose difference can increase while the arterial blood glucose level is decreasing.

The fact that following hemorrhage the glucose A-V difference increased more in

the diabetic dogs than in the non-diabetic dogs may also be explained by the larger rise in the blood glucose level of the diabetic animals, increasing the amount of glucose necessary to establish equilibrium between the blood and tissues and thus enlarging the A-V difference. During periods of rapid fluctuation in glucose concentration the A-V difference may largely reflect attainment of equilibrium. However, in these experiments the glucose A-V difference often remained at high levels for an hour or more with very little change in the arterial blood glucose concentration (table 3, *dog 1*; table 2, *dog 13*). Glucose equilibrium between blood and muscles in non-diabetic rats is reached in three minutes (27). In dogs the volume into which an intravenous dose of 0.75 gm/kg. of glucose must distribute itself five minutes after injection in order to raise the arterial blood glucose concentration the required amount can be calculated (ignoring urine loss and utilization). The results indicate that the glucose has distributed itself in approximately 50 per cent of the total body weight of the dog (3). This value is close to the figure of 65 to 66 per cent of the body weight found by Painter (28) for the total body water of dogs. The fact that five minutes after injection of 0.75 gm/kg. of glucose into control dogs the glucose A-V difference was often positive and was not significantly different from the pre-injection A-V difference (3) furnished additional evidence that glucose equilibrium between blood and tissues is rapidly achieved. It is recognized that glucose does not distribute itself in equal concentrations in all the tissues (27, 29).

Because an increase in the glucose A-V difference occurred in the absence of insulin and because this increase cannot be explained entirely by changes in the blood flow, in the arterial blood glucose level or by the achievement of equilibrium, an additional mechanism must be present which, following hemorrhage, influences glucose utilization. No explanation is offered for the larger glucose A-V difference after bleeding in the depancreatized dogs and the alloxan dogs, as compared with the non-diabetic controls.

Thus, following hemorrhage, glucose disappeared in the periphery of the diabetic dogs at a faster rate than in the control dogs. Furthermore, bleeding the diabetic series increased the arterial blood glucose level more than bleeding the control series. Where is the source of the extra glucose in the diabetic series? As the liver glycogen stores in a diabetic animal tend to be low (30–32), especially following an 18- to 24-hour fast, simple calculation indicates that considerable gluconeogenesis must go on in these diabetic animals to raise and maintain the blood glucose concentration at the high level found. For example, if we assume the pre-hemorrhagic liver glycogen level of the postabsorptive diabetic dog to be approximately one per cent (Fisher and Lackey (30) give a value of 0.05 per cent for dogs on a mixed diet) and the liver to be 3.5 per cent of the body weight (32), in a 10 kg. dog, we would have roughly 3.5 gm. of glycogen in the liver which could be released as glucose after hemorrhage. Distributed in an extracellular volume comprising 25 per cent of the body weight (17), 3.5 gm. of glucose would only be sufficient to increase the arterial blood glucose level 150 mg. per cent providing no glucose disappeared from the extracellular compartment. The average rise in the blood glucose concentration in the alloxan diabetic dogs was 231 mg. per cent and glucose was disappearing rapidly into the peripheral tissues. Loss of glucose in the urine may be disregarded, because urine formation is

at a minimum following severe hemorrhage. Even if there is no increase in gluconeogenesis in diabetes under control conditions (26, 20) a severe stress, such as a hemorrhage consisting of 75 per cent of the bleeding volume, might cause more gluconeogenesis in diabetic than in non-diabetic dogs. It is possible that gluconeogenesis is the source of the extra glucose found in the two diabetic series after bleeding.

Statistically speaking there was no significant difference in the average prehemorrhagic blood lactate and pyruvate values and L/P ratios in the three groups of animals, except for the higher lactate concentration of the depancreatized dogs (table 5). In view of the fact that this series consisted of only 3 animals and that Chesler and Himwich (34) reported no change in the lactate and pyruvate levels of their depancreatized dogs, these elevated lactate concentrations probably should not be considered significant. One hour after bleeding there was no significant difference in the average lactate values in the three series. The two groups of diabetics had a significantly lower pyruvate level and higher L/P ratio than those of the control dogs. Terminally the average lactate, pyruvate and L/P values were similar for the three series of animals.

SUMMARY

1. The glucose metabolism in a series of alloxan diabetic and a series of depancreatized dogs was investigated before and after a 75 per cent hemorrhage (bled out and 25 per cent of the bleeding volume immediately returned) and compared to a series of control animals similarly bled.

2. Glucose utilization before bleeding, as measured by femoral A-V glucose differences, was found to be approximately the same in the two diabetic series as in the control series.

3. Following hemorrhage the average maximum increment in the arterial blood glucose level was twice as large in the alloxan diabetic animals as in the control animals. The arterial blood glucose concentration of the depancreatized dogs after bleeding rose an average maximum value of 52 mg. per cent more than in the control dogs but the difference between the two series was not statistically significant.

4. The rate of disappearance of glucose in the periphery, as measured by femoral A-V glucose differences and blood flows, increased more following bleeding in the alloxan diabetic and depancreatized series than in the control series. Statistically speaking the difference between the diabetic and the non-diabetic dogs was somewhat doubtful in the case of the alloxan animals but was of definite significance in the case of the pancreatectomized animals.

5. Glucose disappeared in the periphery of the depancreatized dogs and perhaps also in the alloxan diabetic dogs at a faster rate than in the control animals following a hemorrhage of similar severity. Furthermore the posthemorrhagic arterial blood glucose level, at least in the alloxan diabetic animals, was raised to a significantly higher concentration than in the control dogs. Hepatic gluconeogenesis was indicated as the probable source of the extra glucose appearing in the diabetic animal after hemorrhage.

6. Lack of insulin made no difference in the general pattern of arterial blood glucose concentration and utilization after bleeding. In both the diabetic and the

non-diabetic groups the arterial blood glucose concentration rose with hemorrhage and there was almost invariably a decrease in the concentration within the last 30 minutes of survival. The glucose utilization also rose to a maximum after bleeding and this maximum was followed by a progressive decrease until death supervened.

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DESOXYCORTICOSTERONE ACETATE AND BLOOD PRESSURE OF DOGS ON A HIGH SODIUM CHLORIDE INTAKE

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THE production of hypertension in patients with Addison's disease during treatment with desoxycorticosterone acetate (DCA) (1–3) has stimulated attempts to produce hypertension in subjects with normal blood pressures by the administration of the hormone (2, 4). Perera and Blood (4) found that the blood pressures of 10 normotensive subjects were not affected by the administration of 5 mg. of DCA given subcutaneously twice daily for one week, with the patients receiving 5 to 10 grams of NaCl orally each day. On the other hand, these investigators (4) found that the blood pressures of 14 subjects with uncomplicated hypertensive vascular disease were increased under the same conditions.

Favorable reports (5) on the treatment of hypertension with a diet poor in sodium have stimulated research in the direction of the adrenal cortical hormones and their possible rôle in the production of essential hypertension.

It has been shown that the parenteral administration of DCA in dogs produces a diabetes-insipidus-like syndrome, i.e., polydipsia and polyuria (6, 7). Selye and associates (8–12) have produced malignant hypertension in rats on a high NaCl intake by the subcutaneous administration of 3 mg. of DCA twice daily for the first month and 5 mg. of DCA twice daily during the second month of treatment. Selye and Hall (8), using 2 recently weaned puppies, administered 5 mg. of DCA subcutaneously twice daily for one week, 10 mg. twice daily for the second week and 20 mg. twice daily for the remainder of the experiment. The female animal received 1530 mg. of DCA over a 47-day period; the male received 2450 mg. over a 70-day period. During the period of administration of the DCA paralysis of the neck and shoulder muscles could be produced by giving the dogs NaCl. The affect on the blood pressure was not noted. Autopsy of these 2 puppies revealed that the kidneys were enlarged and pale, the convoluted tubules were dilated and a proliferation of cells had occurred within the glomeruli. The hearts were enlarged and pale. The adrenal glands, both the cortex and the medulla, showed an extreme degree of atrophy. The liver, pancreas, thyroid, parathyroid and pituitary glands showed no appreciable change grossly or microscopically.

According to Durlacher and Darrow (13), a low potassium diet in the rat causes hypertrophy of the kidney and dilatation, hypertrophy and hyperplasia of the loops

of Henle and of the collecting tubules. Similar though less marked changes were produced by the administration of DCA to rats on a normal diet.

METHOD

Six adult, healthy-appearing, mongrel dogs were used. The dogs received an adequate diet and raw meat twice weekly. All dogs were given a two-month acclimatization period before the experiment was begun. During this acclimatization period the weights were checked daily and blood pressure determinations were obtained two or three times weekly. The method of determining the blood pressure was by direct femoral artery puncture using a mercury manometer.

Two dogs were used as controls, 2 received, in addition to the regular diet, 10 grams of NaCl daily, and 2 dogs received the regular diet, 10 grams of NaCl daily and intramuscular injections of DCA.

Control Animals

Animal no. 5 (male). From February 9, 1948 to May 12, 1948 the weight gradually rose from 44 to 51 pounds. In a total of 18 determinations the blood pressure varied between 115 and 130 mm. Hg, and averaged 125 mm.

Animal no. 6 (male). From March 19, 1948 to May 12, 1948 the weight varied between 45 and 48 pounds. In a total of 18 determinations the blood pressure ranged between 110 and 120 mm. Hg, with a mean of 113 mm.

Animals Receiving 10 Grams of NaCl Daily

The oral administration of NaCl, in tablet form, 10 grams daily, was begun on March 17, 1948 and continued to May 12, 1948.

Animal no. 2 (female). From February 4, 1948 to May 12, 1948 the weight gradually rose from 41 to 47 pounds. In a total of 19 determinations the blood pressure varied between 120 and 130 mm. Hg, and averaged 125 mm.

Animal no. 4 (male). From February 9, 1948 to May 12, 1948 the weight gradually rose from 58 to 64 pounds. In a total of 18 determinations the blood pressure varied between 110 and 130 mm. Hg, with a mean of 115 mm.

Animals Receiving NaCl and DCA

These dogs received 10 grams of NaCl in tablet form daily from March 17, 1948 to May 12, 1948. Each animal also received, by intramuscular injections, the following doses of DCA dissolved in peanut oil: from April 5, 1948 to April 28, 1948, 50 mg. daily; from April 28, 1948 to May 11, 1948, 100 mg. daily; on May 11, 1948 each animal received 500 mg. of the hormone. Each animal received a total of 2000 mg. of DCA over approximately a one-month period.

The only reaction to the hormone that was manifested clinically was that for one day, April 23, 1948, when both dogs developed violent, generalized tremors with weakness of the extensor muscles of the head. During the period of administration of DCA both animals lost their appetite for the dog biscuits and were fed raw meat daily.

Animal no. 1 (female). From February 4, 1948 to May 12, 1948 the weight varied from 34 to 38 pounds. In a total of 18 determinations the blood pressure varied between 115 and 140 mm. Hg. The higher readings (above 130 mm. Hg) were taken on this animal during the period of acclimatization before the injections of DCA were begun. The mean of the blood pressure determinations during the period of DCA treatment was 129 mm.

Animal no. 3 (female). From February 4, 1948 to May 12, 1948 the weight varied from 37 to 40 pounds. In a total of 19 determinations the blood pressure varied between 105 and 125 mm. Hg, averaging 112 mm. during the period of treatment.

SUMMARY

The blood pressures and weight of 2 dogs on a high NaCl intake were not affected by the intramuscular injections of large quantities of DCA given over a period of one month, each animal receiving a total of 2000 mg. of DCA.

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STABILITY OF PROTHROMBIN AND Ac-GLOBULIN IN STORED HUMAN PLASMA AS INFLUENCED BY CONDITIONS OF STORAGE[1]

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T HE realization that normal plasma contains a factor which affects the transformation of prothrombin to thrombin (1–5) has made advisable a study of possible changes in this plasma protein occurring in stored human plasma. Because of the close relationships of this plasma-clotting accelerator substance Ac-globulin to prothrombin a study of prothrombin stability was also under taken.

The methods of analysis for prothrombin, which formed the basis of previous reports on prothrombin stability, are now seen to be sensitive to factors other than prothrombin. Consequently studies previously made on prothrombin concentration were not always accurate. In fact none of the previous reports on prothrombin stability in stored human plasma appear to have truly represented the changes of prothrombin activity that occur. The prothrombin activity was followed in this investigation by means of an improved technic, specific for prothrombin. The results of this method indicate a high degree of stability to be possessed by prothrombin in stored human plasma. Also, the changes in plasma Ac-globulin on storage were followed by means of a method believed to be specific for this coagulation factor. Ac-globulin was found to be less stable than prothrombin and to be more sensitive to various conditions of storage. Indeed, for accurate representation of the stability of the components of coagulation, the conditions of collection and of storage must be clearly defined.

It was found in this study that not only does the nature of the anticoagulant influence the stability of Ac-globulin but so also does the anticoagulant concentration. Moreover, a factor contained in platelets was found to be concerned specifically with the rate of Ac-globulin inactivation in stored plasma. The quantity of this factor present in stored plasma is dependent on such conditions as the centrifugation intensity, used to separate the formed elements from the plasma, and upon the type of container surface utilized for collection and storage.

METHODS

To obtain blood samples for consistent and accurate prothrombin and Ac-globulin analysis it is advisable to reduce to a minimum any possible contamination by tissue juices. Blood was drawn from the antecubital vein of normal human subjects. The syringe containing the first few cc. of blood was removed from the needle and replaced by a fresh syringe containing the anticoagulant. Unless otherwise indicated one part of 1.85 per cent potassium oxalate (0.10 M $K_2C_2O_4 \cdot H_2O$) was added

to seven parts whole blood or two parts of 3.2 per cent sodium citrate (0.109 M $Na_3C_6H_5O. \cdot 2H_2O$) to 23 parts whole blood. When the desired volume of blood had been obtained the needle was removed from the vein and the blood mixed with the anticoagulant in the syringe. This fluid was ejected gently from the syringe into a glass container and placed in an ice bath until centrifugation. The whole blood was centrifuged and the plasma removed, with care taken that none of the precipitated cellular elements be included in the plasma. This plasma was placed in a stoppered glass container and stored in the refrigerator at 3 to 5° C.

Plasma samples to serve as controls were removed at this point and frozen at −20° C. A sufficient quantity for one analysis was placed in each of a number of small test tubes which were stored in a deepfreeze until the time for analysis, when one was removed and thawed. Clean dry glassware was used except in the silicone experiments.

The determination of prothrombin activity was done by two methods. In the first of these, the 2-stage method (6, 7), the plasma is defibrinated by the addition of purified thrombin and the resultant clot removed. This defibrinated plasma is diluted with physiological saline, recalcific in the presence of an excess of thromboplastin and added to a standard fibrinogen solution so that the amount of thrombin formed will give a clotting end point in the approximate range of 15 seconds. The second method was a modification (8) of the 2-stage test previously described. The modification consists merely in substituting bovine serum (diluted 1:600 in the final reaction mixture) for the saline dilution of the defibrinated plasma. This modification is believed to supply sufficient Ac-globulin for full prothrombin conversion. Bovine serum contains a fairly stable and high Ac-globulin concentration (9). Determinations by the unmodified and modified 2-stage methods were made simultaneously.

Ac-globulin concentration was measured by the method of Ware and Seegers (5). The prothrombin, thromboplastin and calcium concentrations are controlled, leaving the rate of thrombin production proportional to the amount of Ac-globulin in the plasma sample to be tested.

<div align="center">EXPERIMENTAL</div>

Prothrombin. To study the stability of prothrombin both citrate and oxalate were utilized individually as anticoagulant mediums. Blood was taken at the same drawing into separate syringes containing the anticoagulants. Centrifugation was carried out at 3000 r.p.m. for 20 minutes and the plasma portion preserved.

In citrated plasmas, as is indicated in figure 1A, the prothrombin activity remained unchanged for 8 to 10 days. Both the modified and unmodified 2-stage methods of prothrombin analysis gave similar results. Yet as storage was prolonged beyond 10 days the prothrombin titer, as determined by the original 2-stage method, began gradually to fall. This is an apparent reduction of the prothrombin level, for it did not occur in the measurements by the modified 2-stage analysis (fig. 1A). The modified 2-stage procedure provides Ac-globulin for complete prothrombin conversion to thrombin. This is not assured in the original method. For this reason we believed that the apparent decrease shown by the original method is due to an altera-

tion of some other constituent in the plasma than prothrombin, presumably Ac-globulin.

A more striking comparison of the two methods was afforded by the change appearing in oxalated plasma. In figure 1B is seen the marked decline in prothrombin activity evidenced by the unmodified 2-stage analysis. However, here again no prothrombin change was seen when an adequate supply of accelerator substance is present as in the improved 2-stage technic.
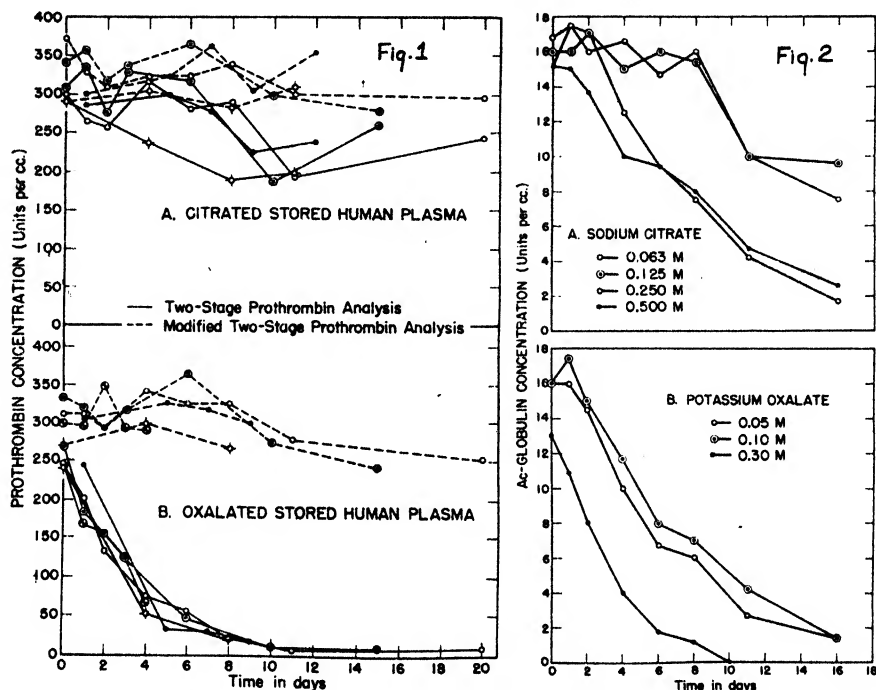
Fig. 1. PROTHROMBIN ACTIVITY in stored human plasmas decalcified with sodium citrate (*A*) and potassium oxalate (*B*) at standard concentrations. Prothrombin determinations were made by the original 2-stage method of analysis and by the modified 2-stage technic.

Fig. 2. EFFECT OF ANTICOAGULANT CONCENTRATION on Ac-globulin stability in stored human plasma. One part of anticoagulant at the concentration given was added to nine parts of whole blood.

It seems pertinent to mention at this point that in the plasma samples which were also followed by means of Quick's 1-stage test (10, 11), a stability difference was observed depending upon whether oxalate or citrate was used as anticoagulant. The observed increase in the prothrombin time in oxalated plasmas agreed with the observations of that author. However, no prothrombin time increase occurred in the citrated plasmas until after the fifth day of storage, and then did not progress so rapidly as the change which had appeared in the oxalated plasma.

In another experiment to be discussed more fully later stability studies were carried out for as long as 56 days (fig. 4) with no alteration in the prothrombin concentration evident when the analyses were done by the modified 2-stage method.

By the old technic the apparent prothrombin titer of oxalated plasma fell to a minimum of 3 to 5 per cent of the original concentration and was maintained at that level. In citrated plasma the decrease did not begin so soon nor was it as marked as that which occurred in oxalated plasma.

Thus it is evident that there is a difference in the stability of the stored coagulation factors. The activity of the less stable protein would seem to depend upon whether citrate or oxalate is utilized to maintain the plasma in the fluid state. Moreover, these alterations are not attributable to changes of prothrombin itself for, if the activity of this plasma fraction is accurately measured such as by the modified 2-stage technic, no decrease was found in 56 days under storage conditions in either citrated or oxalated human plasma.

To further examine the reason for the discrepancy between the original and the modified 2-stage procedure the following experiment was undertaken. An oxalated sample of human plasma stored in the refrigerator for 53 days and containing no detectable Ac-globulin was used. Prothrombin determinations were carried out by the original and by the standard modified 2-stage procedure. In addition, tests were

TABLE 1. PROTHROMBIN ACTIVITY IN OXALATED HUMAN PLASMA STORED 53 DAYS

METHOD OF PROTHROMBIN ANALYSIS	PROTHROM-BIN CONCEN-TRATION[1]
	U/cc.
1. 2-stage unmodified	19
2. 2-stage modified by use of bovine serum	288
3. 2-stage modified by use of purified Ac-globulin	295
4. 2-stage modified by use of bovine platelet extract	285

[1] Original prothrombin concentration = 290 u/cc.

carried out by modifications of the 2-stage method in which a purified serum type Ac-globulin (9) or a potent bovine platelet extract was substituted for bovine serum as the diluent for the defibrinated plasma. As can be seen in table 1 the very low value found by the original technic is in sharp contrast to the full prothrombin titer that was achieved with all three of the modifications of the prothrombin test. Whether bovine serum or a purified product is the source of Ac-globulin does not appear to be of importance. Nevertheless the concentration of accelerator factor must reach at least a certain level or the conversion will be incomplete, and an inaccurate, low-prothrombin value will be obtained. A factor in platelets which affects prothrombin conversion to thrombin has recently been studied extensively in this laboratory. This factor is described as having much the same function as Ac-globulin. Here is demonstrated, with the use of stored plasma, the ability of the platelet factor to accelerate and cause complete conversion of prothrombin to thrombin.

Ac-globulin. The study of the stability of coagulation factors in human plasma was extended to Ac-globulin. Here again both oxalated and citrated plasmas were used (fig. 3A). Ac-globulin was found to be relatively stable in citrated plasma, maintaining full potency for more than a week before entering upon a period of gradual decline. Oxalated human plasma on the other hand did not seem to provide

a suitable medium for preservation of Ac-globulin activity.   Thus at the concentrations used oxalate and citrate differ in their affect on the stability of what appears to be a single plasma fraction, Ac-globulin.   The similar results obtained with the Ac-globulin assays and the unmodified 2-stage method of prothrombin analysis (fig. 3 and 4) substantiate further the conclusion that the latter test is sensitive to a fall in Ac-globulin when the human prothrombin/Ac-globulin ratio passes a certain critical value.

Anticoagulant Concentration.   Ac-globulin sensitivity in stored human plasma to the factors affecting its stability indicate that these conditions needed to be further defined.   In the previous studies 0.109 M sodium citrate was combined with whole blood in the ratio of two parts to 23.   When potassium oxalate was used as anticoagulant one part of a 0.10 M solution was added to seven parts of whole blood.   To further investigate the effects of these decalcifying agents sodium citrate was prepared in 0.50, 0.25, 0.125 and 0.063 M concentrations and potassium oxalate was made up in 0.30, 0.10 and 0.05 M solutions.   To 1 volume of each anticoagulant concentration were added 9 volumes of blood, all samples being obtained at one drawing of blood.   After centrifugation for 30 minutes at 3000 r.p.m. the plasma was removed and stored in the refrigerator.   The stability of Ac-globulin as observed in these plasmas is graphically represented in figure 2.

This is additional evidence that Ac-globulin is more stable in citrated than in oxalated plasma depending, however, on the concentration of anticoagulant used. It is evident that at the lower concentrations the Ac-globulin is more stable in both mediums.   Interestingly enough the plasma stability curves from the bloods mixed with the higher concentrations of sodium citrate can be superimposed upon those obtained when small amounts of potassium oxalate were used without any identifying difference being discernible.   The evidence indicates that for optimal Ac-globulin stability in human plasma the concentration range for these anticoagulants is low. No evidence is presented in this study to explain the mechanism of the difference between the results obtained with citrate and with oxalate.

Silicone and Glass Surfaces.   Surface phenomena have long been appreciated as a factor involved in coagulation.   By the use of silicone surfaces Jaques *et al.* (12) have shown that this nonwetting surface will extend the clotting time of native blood and plasma.   Platelets do not agglutinate nor do they disintegrate so rapidly when the blood is in contact with silicone as compared to glass.   In order to study the effect of surfaces upon prothrombin and Ac-globulin activity in stored plasma the following experiment was carried out.   Whole blood was taken, centrifuged 15 minutes at 2000 r.p.m. and the plasma removed and stored, utilizing in one case siliconed surfaces throughout and in the other the usual glass.

The type of surface appears to have no effect upon prothrombin stability (fig. 4).   However, the fall in activity by the original 2-stage prothrombin technic was retarded in the citrated plasma collected in silicone containers.   The Ac-globulin level in citrated plasma fell more completely when in contact with the glass surface as compared to silicone (fig. 3).   Oxalate appears to have altered the Ac-globulin so rapidly in this experiment that the possible effects of surface are not apparent.

Because most of the formed elements are removed at the intensity of centrif-

ugation employed in this experiment and, also, considering the lack of a marked alteration in stability of the factors studied, it seems reasonable to conclude that
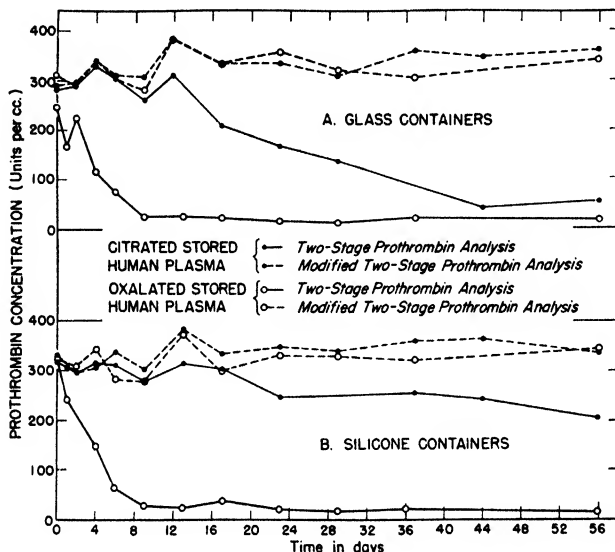
Fig. 3. PLASMA Ac-GLOBULIN CONCENTRATION in glass (*A*) and siliconed (*B*) containers. Plasma obtained by standard methods.
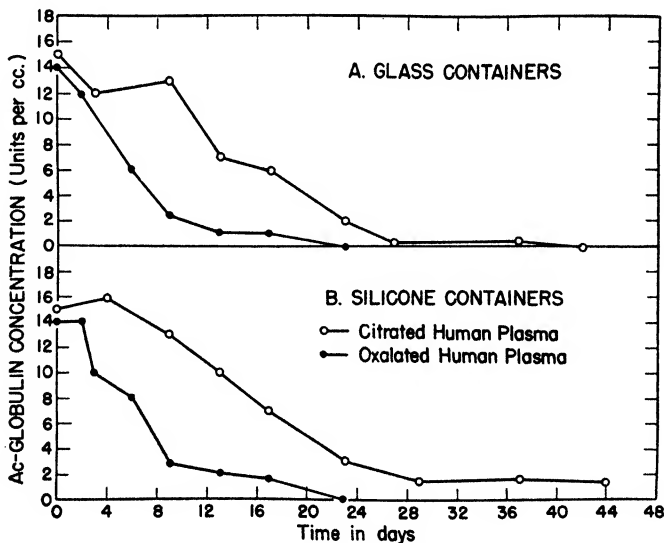
Fig. 4. PROTHROMBIN CHANGES in glass (*A*) and siliconed (*B*) containers on storage of human plasma. Determinations were carried out by the original and by the modified 2-stage procedure. Standard anticoagulant concentrations used.

there is no direct difference in the effect of a wetting and nonwetting surface upon the prothrombin or Ac-globulin stability of a stored plasma. However, presumably

in a glass container more platelet disintegration would occur than in a siliconed container before separation of the plasma from the cellular elements. A plasma in contact with glass when finally removed from above the precipitated cellular elements would contain a considerable amount of platelet breakdown products in comparison with the plasma taken from a siliconed container where platelet destruction was minimized. Thus the small difference in Ac-globulin stability within the two types of
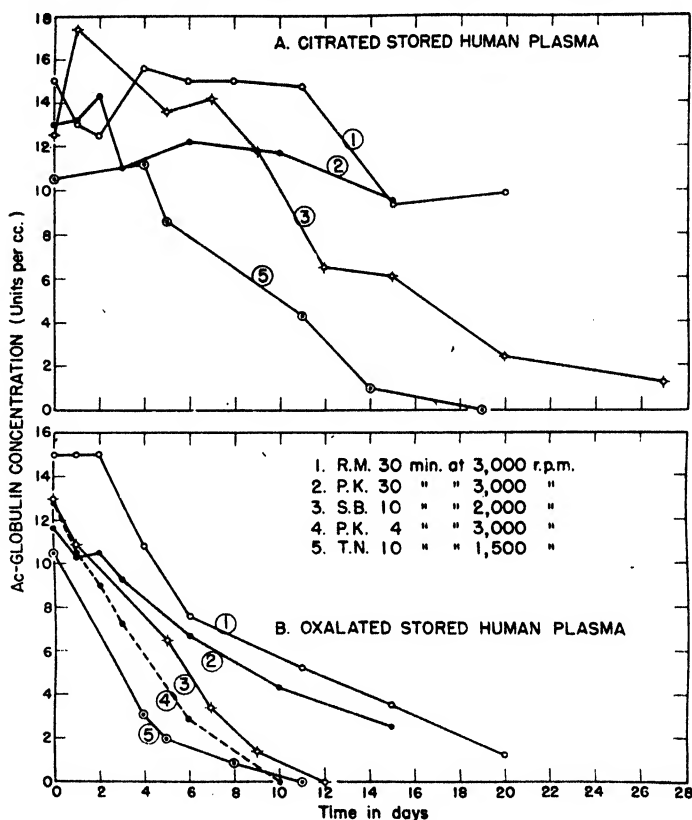
Fig. 5. INFLUENCE OF CENTRIFUGATION INTENSITY on Ac-globulin stability in stored citrated (*A*) and oxalated (*B*) human plasma. *Plasmas 2 and 4* were obtained from the same drawing of whole blood. Whole blood was collected under standard conditions.

surfaces becomes of possible significance and would indicate that this might be due indirectly to the effect of these surfaces on the formed elements of the blood.

Centrifugation. In the course of these studies it was observed that the degree of centrifugation seemed to affect plasma Ac-globulin stability. Both oxalated and citrated human bloods were, therefore, subjected to rates and duration of centrifugation varying from 5 minutes at 1500 r.p.m. to 30 minutes at 3000. The plasma was then removed and stored. With increasing intensity of centrifugation and precipitation of more cellular elements the plasmas progressed from translucent to clear with respect to transmitted light. Platelets are the suspended elements involved as the minimal centrifugation removed most of the erythrocytes.

Ac-globulin stability decreases as the intensity of centrifugation is reduced (fig. 5). The evidence seems clearly to implicate platelets among those factors which influence the stability of Ac-globulin in stored plasma, the presence of platelets having an adverse effect upon maintenance of Ac-globulin activity.

Platelet Factor. To further study the effect of platelets upon the stabilizing ability of plasma an extract was prepared by differential centrifugal separation of the platelets, trituration and extraction with saline as described in an accompanying paper. To 3 volumes of fresh citrated human plasma which had undergone relatively hard centrifugation (20 minutes at 4500 r.p.m.) to remove the cellular elements was added 1 volume of the platelet extract or saline. The level of plasma Ac-globulin was followed under refrigerated storage conditions and the results charted in figure 6.
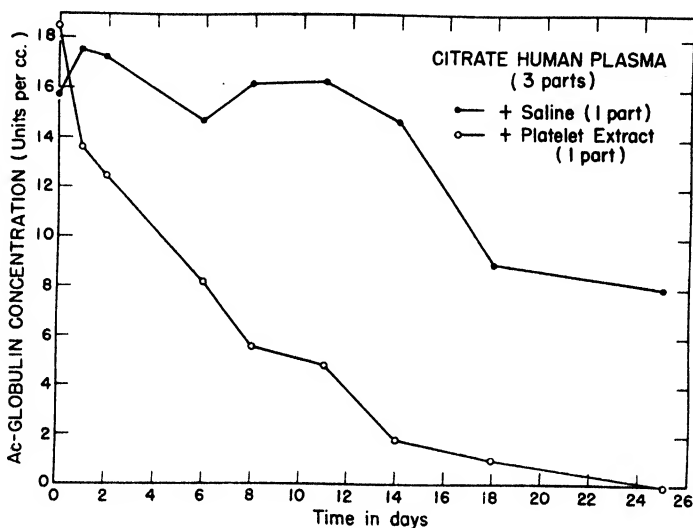
Fig. 6. EFFECT OF PLATELET EXTRACT on Ac-globulin stability in stored human plasma decalcified by a standard sodium citrate concentration.

The initial high level in the plasma found one-half hour after mixing with platelet extract is to be expected because of the existence in platelets of a factor previously referred to which accelerates the prothrombin conversion rate and which is additive to the plasma Ac-globulin activity. By the end of 24 hours the inhibitory effect became manifest and the Ac-globulin titer was less in the platelet extract plasma than in the plasma with saline alone added. On continuation of storage the instability of Ac-globulin became more marked in the medium containing platelet products. This experiment has been repeated and confirmed in citrated and oxalated human plasmas. Furthermore, the quantity of platelet extract comparable to that obtainable from a physiological platelet concentration (13) causes a distinct decrease in Ac-globulin activity.

From these results it is evident that platelets contain a factor which decreases the stability of Ac-globulin in decalcified stored human plasma. This property may

be overshadowed initially by the activity of the accelerator liberated from the platelets. The inhibitory activity is manifest whether oxalate or citrate is used as anticoagulant.

Further Studies on Prothrombin. Table 2 summarizes the effects of the various storage conditions upon prothrombin. When storage was carried beyond two months the prothrombin level began to fall. Bacterial action may have been responsible for this alteration. Different concentrations of anticoagulants in the range studied seem to have no influence on the maintenance of prothrombin activity. Also the platelet factor appears not to be of importance in the stability of prothrombin in stored plasma. From this data and that recorded earlier the high stability of prothrombin

TABLE 2. EFFECTS OF VARIOUS STORAGE CONDITIONS UPON PLASMA PROTHROMBIN ACTIVITY AS MEASURED BY THE MODIFIED TWO-STAGE METHOD

A. SILICONE AND GLASS				B. ANTICOAGULANT CONCENTRATION		C. PLATELET EXTRACT
Plasma	Days stored			Concentration of anticoagulant added to blood	Prothrombin after 30-day storage	Prothrombin after 39-day storage
	56	80	116			
	Prothrombin				*U/cc.*	*U/cc.*
	U/cc.	*U/cc.*	*U/cc.*			
Silicone				Sodium citrate		
Citrate	335	220	140	0.063 M	333	314[1]
				0.125 M	333	
Oxalate	345	314	182	0.250 M	329	340[2]
				0.500 M	322	
Glass				Potassium oxalate		
Citrate	341	270	181	0.05 M	333	320[3]
Oxalate	330	285	174	0.10 M	300	341[4]
				0.30 M	310	

[1] Sample prepared by mixing 3 parts citrated plasma with 1 part platelet extract before storage
[2] Sample prepared by mixing 3 parts oxalated plasma with 1 part platelet extract before storage
[3] Control: 3 parts citrated plasma mixed with 1 part saline before storage.
[4] Control: 3 parts oxalated plasma mixed with 1 part saline before storage.

in stored human plasma stands in contrast to the variable but more rapid decrease in activity shown by Ac-globulin.

DISCUSSION

The conditions of collection and storage have an important relation to the stability of plasma coagulation factors. Determination of prothrombin and Ac-globulin concentrations by improved and specific methods show prothrombin to be quite stable, whereas Ac-globulin is much more sensitive to the conditions imposed upon the stored plasma. The presence of platelets or platelet extracts accelerates the rate of Ac-globulin inactivation. The effects of various surfaces and centrifugation intensities appear to exist because of their relation to the platelet content of plasmas. In addition, Ac-globulin is less stable in potassium oxalate than in sodium citrate at equimolar concentrations and less stable in both anticoagulants at higher concentrations.

In earlier reports on plasma or whole blood it is not always clear what the conditions of storage were.   Also, the methods of analysis were not specific.   The changes formerly attributed to prothrombin in stored human plasma were not due to alterations in the prothrombin itself, but were the result primarily of the change occurring in the separate plasma protein, Ac-globulin.   Fibrinogen may or may not have been involved in results dependent upon the 1-stage method of analysis (14–17).   Variations in fibrinogen do not effect results obtained by the 2-stage technic.

In general the changes measured by means of the 1-stage test indicated prothrombin to be unstable (18–22), but not all the reports agreed (23–25).   Those groups of workers that have used both the 1-stage and the 2-stage methods of analysis report fairly high prothrombin stability by the 2-stage method.   Ziegler, Osterberg and Hovig (26) feel that the correlation between the two methods is rather good. Lord and Pastore (27) on the other hand indicate considerable discrepancy.   Both utilized bank blood.   Warner, DeGowin and Seegers (28) found by the 2-stage technic that 50 per cent activity remained after three weeks in stored citrated plasma. A somewhat greater loss occurred by the 1-stage determinations.

In 1943 Quick (29) found that upon mixing stored human plasma and fresh dicumarol dog plasma an unexpectedly high prothrombin value was obtained by the 1-stage test.   On the basis of this evidence he stated that upon storage of decalcified human plasma one factor in the thrombin-forming mechanism is labile while another remains stable.   These he named, respectively, *component A* and *B* of prothrombin, and postulated that prothrombin is composed of these two essential and separable factors combined with calcium.   Soon thereafter Seegers, Loomis and Vandenbelt (30) predicted a reinterpretation of Quick's data for, on the basis of extensive prothrombin purification work, they found no evidence for more than one component of prothrombin.

As early as 1939 Smith and his associates recognized variability in the prothrombin to thrombin conversion rate (31–33).   This was encountered particularly in comparison of the times required for thrombin production in various species. These differences were attributed to a 'convertability factor of unknown nature' (34). In working with purified prothrombin Mellanby (35) and Seegers (36, 37) noted slow activation of prothrombin to thrombin.

More recently Owren (1, 2), Fantl and Nance (3, 38) and Ware, Guest and Seegers (4, 5) independently identified a substance in normal plasma which is necessary for the physiological transformation of prothrombin to thrombin.   This factor was named accelerator globulin (Ac-globulin) by these last investigators because of its chemical and functional properties.   It appears to be the same as Owren's *factor V* and the accelerator substance of Fantl and Nance.   Honorato (39), referring to a plasmatic cofactor of thromboplastin to distinguish it from prothrombin, also appears to be dealing with this same substance.   Further, from the evidence presented here and otherwise now known about plasma Ac-globulin it may be assumed that this factor is that with which Quick was primarily concerned when he first postulated the *component A* of prothrombin.

A modification of Quick's original concept and terminology was made by him in 1947 (40).   *Component B* (prothrombin) was recognized as the compound or frac-

tion which is the true mother-substance of thrombin. The term *component A* was replaced by 'labile factor' as a name for the substance which decreases in activity in stored oxalated human plasma. Though not described as an accelerator 'labile factor' presumably applied to that plasma fraction referred to here as Ac-globulin. Actually Ac-globulin is stable in oxalated beef plasma (41) and in citrated human plasma when stored at refrigeration temperature. Only under certain conditions is this factor found to be unstable.

SUMMARY

The stability of prothrombin and of Ac-globulin has been studied in normal human plasma under various conditions of collection and of refrigerated storage. To accurately measure the level of prothrombin activity under storage conditions a modification of the original 2-stage method is required in which sufficient accelerator substance is provided for full conversion of prothrombin to thrombin. Prothrombin was found to be stable in oxalated or citrated stored human plasma.

Ac-globulin is sensitive to the conditions under which the experiment is carried out. At equal molar concentrations of anticoagulants the Ac-globulin is found to be less stable in oxalated than in citrated human plasma. At a high citrate concentration the Ac-globulin titer may fall as rapidly as in a lightly oxalated plasma. Silicone and glass surfaces exert no direct influence on Ac-globulin stability. However, the type of surface by means of its effect upon platelet stability indirectly is related to the control of plasma Ac-globulin stability. The degree to which platelets are separated from the plasma by varying the centrifugation intensity affects the amount of alteration in Ac-globulin on storage. Plasma is found to be a more stable medium when the platelet content is low. Addition of a platelet extract to decalcified human plasma decreases the plasma Ac-globulin stability.

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CONCENTRATION OF PROTHROMBIN AND Ac-GLOBULIN IN VARIOUS SPECIES[1]

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EVIDENCE has recently been reported indicating that plasma contains a factor which accelerates the rate of conversion of prothrombin to thrombin (1-5). Ware, Guest and Seegers (4) have termed this factor Ac-globulin since it is a globulin and since it accelerates the interaction of prothrombin and thromboplastin in the presence of calcium ions. Evidence has also been presented which indicates that Ac-globulin exists in two forms (6). The enzyme found in plasma (plasma Ac-globulin) is probably a proenzyme while that found in serum (serum Ac-globulin) is the active catalyst. It has been shown, using purified reagents, that thrombin is capable of activating the proenzyme (6, 7). As an explanation of the clotting reactions it has been postulated (6), therefore, that prothrombin interacts first with thromboplastin in the presence of calcium ions to form thrombin slowly. The thrombin in turn causes the change in activity of plasma Ac-globulin to serum Ac-globulin. The latter then intensifies the interaction of prothrombin and thromboplastin so that thrombin is formed rapidly. Fibrinogen is then converted to the fibrin clot through the action of thrombin.

Variations in the concentration of Ac-globulin could reasonably be expected to influence markedly the 1-stage prothrombin times (8) since this procedure measures the rate of thrombin formation (8). On the other hand, such an influence should be partially eliminated in the 2-stage method of prothrombin analysis of Warner, Brinkhous and Smith (9) since in this method the prothrombin is allowed to convert to thrombin before analysis is made. That some such factor is involved in these methods of assay is suggested by the marked discrepancies in the results reported by the two methods. Warner, Brinkhous and Smith (10) report the following prothrombin levels obtained by the 2-stage method of analysis: dog 100, rat 95, cat 91, rabbit 89, man 84, guinea pig 53, chicken 50, turtle 42. Quick (11), using the 1-stage method of analysis, found the following prothrombin relationships: dog 100, rabbit 100, cat 60, man 20 and cow 16. Warner, Brinkhous and Smith (10, 12) explained this discrepancy by postulating a 'convertability factor'. These workers noted that man and guinea pig prothrombin was converted slowly to thrombin in the 2-stage assay procedure, while dog and rabbit were 'rapid converters'. This offered an explanation for the relatively low prothrombin values for man and the high values of dog and rabbit reported by Quick (11). Other discrepancies between the 2-stage and the 1-stage methods have also been reported and explained on the basis of the convertability factor (13-15). If the original hypothesis concerning a convertibility factor is correct one might expect a high Ac-globulin activity in dog and rabbit and lower activity in guinea pig and man. Partial purification and development of a quantitative method of analysis for Ac-globulin (16) has made possible a study of the concentration of this factor in these various species.

EXPERIMENTAL

Methods. Blood was obtained from stock laboratory animals chosen at random. The blood was collected by arterial cannulation or by cardiac puncture and was

mixed with 1.85 per cent potassium oxalate in the respective ratio of 7 to 1. A portion of the blood from each animal was permitted to clot spontaneously in clean dry test tubes. Cell free serum and plasma were obtained by centrifugation at 3000 r.p.m. for 30 minutes. All samples were kept at 5°C. until analyzed. Hematocrits were obtained on all specimens and all values reported are corrected for anticoagulant dilution.

Prothrombin analyses were performed by the 2-stage method of Warner, Brinkhous and Smith (9). Ac-globulin determinations were done according to the procedure described by Ware and Seegers (16). Purified prothrombin for Ac-globulin analyses was prepared according to methods of Ware and Seegers (17). To minimize variations in reagents all analyses were standardized to controls. These controls consisted of bovine plasma and bovine serum which were stored at −20°C.

Plasma Ac-globulin Concentrations. The results of the analyses of the plasmas of various species for Ac-globulin are shown in table 1. Significant differences in activity are seen. Of the mammals studied, man and guinea pig have the lowest Ac-globulin concentration while dog and rabbit have the highest, cow being intermediate. Some variation was noted in the type of prothrombin activation curves obtained with plasmas from the different species. This was especially true in the case of the rabbit plasma and may be a possible explanation of the variation in Ac-globulin concentration found with different samples from this species. Similar difficulty was also experienced in the interpretation of the activation curves produced by the rat, chicken, and turtle plasmas.

Prothrombin Concentration. Prothrombin analyses were also performed on the same plasmas and the results are shown in table 1. With the exception of the dog these results correlate fairly well with those obtained by Warner, Brinkhous and Smith (10). Repeated analyses have shown the prothrombin of the dog to range between 180 and 200 units per cc. while Warner has reported higher values for this species. The reason for this discrepancy is not clear.

Inasmuch as Ac-globulin has been shown to affect not only the rate of thrombin formation but also the thrombin yield (4, 5), it occurred to us that perhaps in some species a lack of Ac-globulin might be limiting the final thrombin yield and therefore giving too low a prothrombin value when measured by the 2-stage method. To test this possibility an optimum amount of Ac-globulin was supplied in the 2-stage method. For this purpose prothrombin-free bovine serum was supplied in the 2-stage method so that it was diluted 800 times in the final clotting mixture. The results are shown in table 1. It is evident that no outstanding difference was noted in the prothrombin values obtained by the 2-stage method of Warner, Brinkhous and Smith and by the modified method (18).

Serum Ac-globulin. Analyses of serum for Ac-globulin activity are shown in table 1. The data indicate that the values for the serum showed marked variation in activity from what might be expected when compared to homologous plasma values. Human and dog serums showed marked loss of Ac-globulin activity as compared to bovine. It was found that if human or dog blood was centrifuged for 10 minutes immediately after withdrawal, serum Ac-globulin activity of comparable degree to plasma could be demonstrated. Furthermore, it has been shown that on storage there is a rapid loss of Ac-globulin activity in these sera. Rabbit serum is

more like beef serum in that its Ac-globulin activity is relatively stable. In some instances as much as 50 per cent of the activity remained at the end of 24-hour storage at room temperature.

TABLE I. CONCENTRATION OF PROTHROMBIN, PLASMA AC-GLOBULIN AND SERUM AC-GLOBULIN FOR VARIOUS SPECIES

SPECIES	PROTHROM-BIN (2-stage)	PROTHROM-BIN (2-stage, mod.)	PLASMA AC-GLOBU-LIN	SERUM AC-GLOBU-LIN[1]
	U/cc.	*U/cc.*	*U/cc.*	*U/cc.*
Cat	281	281	170	91.2
	253	253	123	60
	271	302	127	99
Dog	210	210	167	<1
	190	200	203	<1
	205	215	158	<1
Guinea pig	212	249	38	10.8
	203	212	40	<1
	210	214	31	<1
Rabbit	239	239	121	67
	249	249	142	233
	221	215	92	233
		265	310	
Ra	320	330	73	<1
	322	342	55	<1
	323	333		
Chicken	110	118	3.2	<1
	95	97	4.0	<1
	93	98	4.7	
Turtle[2]	68	70	3.0	<1
Human[2]	290	290–315	12–17	<1
Bovine[2]	250–285	250–285	120–140	70–90

[1] At room temperature, and 30 minutes after clotting in ordinary clean glassware.

[2] Pooled: 4 turtles; 12 humans; 15 cows.

Comparison of Plasma Ac-globulin and Serum Ac-globulin. The original investigations concerning the inactive form of plasma Ac-globulin and its change to active serum Ac-globulin as the result of the action of thrombin were made with media of bovine origin (7). The Ac-globulin activity in the sera of man and dog is so unstable it is possible to demonstrate the change in activity from plasma Ac-globulin to serum Ac-globulin only under specially controlled conditions. Therefore, the following experiment was performed in order to demonstrate the change of activity of plasma Ac-globulin to serum Ac-globulin in human blood.

Blood was obtained by venipuncture using syringes and glassware which had been treated with dri-film according to the technic of Jaques *et al.* (19). No anticoagulant was used. The blood was centrifuged at 20,000 r.p.m. for one hour at 5°C. and the plasma, which was free of fibrin, was separated from the cells by the technic described by Patton, Ware and Seegers (20). The plasma was then analyzed for Ac-globulin activity. At room temperature coagulation of the plasma occurred slowly and the fibrin was removed. Analyses were then repeated on the serum under identical conditions. The curves in figure 1 represent the activation of purified bovine prothrombin by the Ac-globulin from the serum and plasma sources in the presence of thromboplastin and calcium ions. Curves *A* and *B* represent the activa-
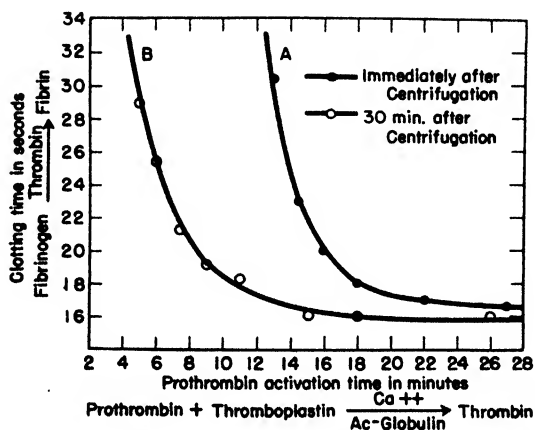
Fig. 1. ACTIVATION OF PURIFIED PROTHROMBIN SOLUTION with excess thromboplastin, optimum calcium ion concentration. Sources of Ac-globulin were *a*) human plasma collected with silicone technic diluted 1500 times and *b*) the same human plasma after slow spontaneous clotting and also diluted 1500 times.

tion of the prothrombin in the presence of Ac-globulin from human plasma before and after clotting had occurred. In each case the human serum and human plasma was diluted 1500 times in the final clotting mixture. It is evident, therefore, that a similar relationship exists between plasma and serum Ac-globulin of human origin as reported for bovine origin (6, 7). However, because of the instability of human serum Ac-globulin this relationship may be easily overlooked. If small amounts of thrombin, for example, about 2 to 5 u/cc., are added to human plasma the same change in the activity of Ac-globulin can be observed. A similar relationship between plasma Ac-globulin and serum Ac-globulin has been demonstrated with the plasmas of dog, rabbit and guinea pig.

DISCUSSION

The findings of plasma Ac-globulin concentrations of 150 to 200 u/cc. for dog, 12 to 17 u/cc. for man, and 30 to 40 u/cc. for the guinea pig offer adequate experimental support to the contention of Warner, Brinkhous and Smith (10, 12, 15) that the difference in prothrombin analyses in various species between the 1-stage and 2-stage methods is attributable to a factor which influences the rate of conversion

of prothrombin to thrombin.   The prothrombin values of the mammals studied fell
in the range of 200 to 300 U/cc. while the Ac-globulin concentration varied between
15 units for man to 350 units for rabbit.   On the other hand, if the 1-stage method
is utilized, the values for dog and rabbit are five times as great as for man.   It would
appear, therefore, that the concentration of Ac-globulin has a profound influence
on the determination of prothrombin by the 1-stage method.   Owren's description
(3) of a syndrome characterized by a deficiency of a factor which we believe to be
identical with Ac-globulin, resulting in an apparent prothrombin deficiency as
measured by the 1-stage method, emphasizes the need for further consideration of
the problem.

Evidence has been reported from other laboratories which substantiates the
findings presented here.   Owren (2) has reported the activity of Factor V to be
greater in bovine and guinea pig plasma than in human plasma.   Fantl and Nance
(21) have shown that if human plasma is diluted with plasmas of various species
from which the prothrombin has been removed by aluminum hydroxide or barium
carbonate, shorter coagulation times result.   Rabbit plasma causes the shortest
coagulation time followed by dog and then guinea pig plasma.

In the bovine species both Ac-globulin and prothrombin values are relatively
high (table 1).   Curiously, Quick (11) reported low prothrombin values in this
species.   This discrepancy suggests that some factor other than Ac-globulin might
be involved.   The question of homologous reagents in species specificity cannot be
ignored.   The data available do not offer an adequate explanation for the discrep-
ancy.

The use of a modified 2-stage method of prothrombin analysis has indicated
that the original 2-stage method of Warner, Brinkhous and Smith is unaffected by
increased amounts of Ac-globulin.   It has been shown, however, that the 2-stage
method also indicates apparent losses of prothrombin activity where exceptionally
low Ac-globulin concentrations are involved (2, 4, 5).   In such instances only the
modified 2-stage technic gives reliable results.   It is apparent, therefore, that some
data obtained with the 2-stage technic may need to be reinterpreted.   In work with
purified prothrombin serious difficulty was encountered (17).   Furthermore, Owren
(3) showed that there is not sufficient Ac-globulin in parahemophilia to insure the full
prothrombin titer.   Experiments on storage plasma, currently being conducted in
this laboratory, also show that a lack of Ac-globulin may effect the 2-stage method.
It is not at once apparent why turtle plasma can be analyzed by either the original
or modified procedure since its plasma contains only 3 U/cc. of Ac-globulin.   It must,
however, be realized that there is as yet no adequate information on the relative
amounts of Ac-globulin which must be associated with prothrombin if the 2-stage
method for prothrombin analysis is to be unaffected.   In the turtle the ratio of pro-
thrombin/Ac-globulin is 70/3 and in man about 300/12.   The human species, there-
fore, has fewer Ac-globulin molecules for each prothrombin molecule than the turtle;
in fact, fewer than any other species studied.   On this basis it becomes apparent that
a reduction of Ac-globulin below normal in man will markedly increase the ratio of
prothrombin to Ac-globulin.   This concept may be of considerable consequence.
The low Ac-globulin activity of human plasma, and a high prothrombin/Ac-globulin
ratio, suggests that a narrow margin of safety exists.   If loss of Ac-globulin activity

occurs in diseased conditions, the effect on the coagulation mechanism may be serious. Furthermore, we anticipate that the ratio of prothrombin/Ac-globulin will be of fully as much significance as a knowledge of the absolute concentration of either of these factors. This idea was already implied in the work of Smith and his associates when they spoke of compensatory mechanisms for low prothrombin values (13, 14).

In most species, including man, serum Ac-globulin is not very stable. The cow and the rabbit are outstanding exceptions, but the reason is not clear. The ultimate cause of instability will probably be ascribed to the action of thrombin. Certainly it has been demonstrated that thrombin in sufficient concentration will destroy Ac-globulin (7). It does not seem necessary to postulate the existence of a special inhibitor of serum Ac-globulin, as has already been suggested by Owren (22).

SUMMARY

Analyses of plasmas of selected species indicate that the relative concentration of plasma Ac-globulin, in units per cc., is as follows: man 12 to 17, dog 150 to 200, cat 130 to 170, rabbit 150 to 300 and guinea pig 30 to 40. The conversion from plasma Ac-globulin to serum Ac-globulin has been shown to take place in human, dog, rabbit, guinea pig and cow plasma; however, the stability of serum Ac-globulin varies considerably in the species studied. In man and dog serum Ac-globulin is extremely labile while in bovine and rabbit serum the factor is much more stable. The reported differences in the concentrations of Ac-globulin in the various species offer at least a partial explanation for the reported discrepancies of prothrombin values obtained by the 1-stage and the 2-stage methods, the latter, when modified, giving the correct values.

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PLATELET EXTRACTS, FIBRIN FORMATION AND INTER-ACTION OF PURIFIED PROTHROMBIN AND THROMBOPLASTIN[1]

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IT HAS been generally accepted that blood platelets accelerate the blood co-agulation process by supplying thromboplastin. Two groups of investigators, however, recently reported that platelets do not supply much thromboplastin but affect the coagulation process in other ways (1, 2). Mann, Hurn, and Magath (1) demonstrated that platelet extracts restore to normal the delayed prothrombin time of stored human plasma only when additional thromboplastin is also provided. They conclude that platelets supply very little thromboplastin but that they poten-tiate the action of tissue thromboplastin. Quick (2) reports that platelets accelerate the clotting reaction not by supplying thromboplastin but by furnishing a substance which changes an inactive form of thromboplastin to active thromboplastin.

The availability to our laboratory, in purified form, of a number of the principal factors which participate in the clotting reactions offered opportunity for elucidating further the exact rôle of platelets in blood coagulation. The results show that platelets contain only small amounts of thromboplastin. Two other substances are present. One is an accelerator which catalyzes the change of prothrombin to thrombin by thromboplastin and calcium ions. It acts like serum Ac-globulin. The second substance, like acacia (3) and some other colloids, hastens the action of thrombin on fibrinogen. We have found no previous reference to this factor and in this paper it is referred to as platelet factor 2.

EXPERIMENTAL

Preparation of Platelet Extracts. Platelet extracts were prepared oxalated from bovine blood by fractional centrifugation. At the slaughter house 350 cc. of blood were collected from a stab wound and mixed immediately with 50 cc. of 1.85 per cent potassium oxalate solution. Within one hour after collection the blood was centrifuged at room temperature at 1500 r.p.m. until most of the red cells had settled. The plasma, which still contained many red cells, was withdrawn and centri-fuged at 0° C. in an angle head centrifuge at 4500 r.p.m. for 20 minutes. The platelets and red cells packed at the bottom of the tube were then resuspended and thoroughly mixed with 30 cc. of saline. Red cells were mostly removed from this solution by light centrifugation at room temperature and the suspended platelets were removed by further centrifugation for 15 minutes at 3000 r.p.m. in conical tubes. The supernatant fluid was discarded and the platelet mass was carefully removed from the remaining red cells packed at the bottom of the tube. When these platelet concentrates were examined on slides prepared with Wrights' stain about 500 platelets were found for each erythrocyte.

Extracts of the platelet concentrates were prepared by thorough trituration in a mortar with a

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small amount of saline. Additional saline was then added to bring the volume of the extract from 0.2 cc. of centrifuge packed platelets to 5 cc. The macerated platelet fragments were removed from the extract by centrifugation at 3000 r.p.m. for 15 minutes.

Preparation of Prothrombin. Prothrombin was prepared from bovine plasma by the most recently described methods (4). The last traces of Ac-globulin were removed by heating for two hours in water solution at 53° C.

Preparation of Thromboplastin. Two hundred cc. of crude thromboplastin prepared from bovine lung (5) were centrifuged 30 minutes at 45,000 r.p.m. in a Sharples Super Centrifuge. The precipitate was resuspended in 200 cc. saline, resedimented, resuspended in 25 cc. saline and centrifuged at 2000 r.p.m. for 5 minutes to remove gross particles. The thromboplastin remaining in the supernatant fluid is very active and free of Ac-globulin (4).

Preparation of Fibrinogen. The detailed procedure has already been described (6). Briefly, oxalated bovine plasma is frozen, allowed to thaw slowly, and the fibrinogen remaining insoluble at 2° C. is harvested by centrifugation, thorough washing and solution in saline. The product was used only when the total protein was at least 95 per cent coagulable with thrombin.

Preparation of Thrombin. Thrombin was prepared from purified prothrombin by a variety of procedures employed while studying methods for obtaining highly purified thrombin. This work is not complete as yet and a detailed description will very probably be offered for publication at a later date. All preparations used had a specific activity equal to or greater than those described by Seegers and McGinty (7).

Analysis for Prothrombin. Both the unmodified two-stage method of Warner, Brinkhous and Smith (5) and the modified procedure of Seegers and Ware (8) were used. The modification consists of the addition of serum Ac-globulin (diluted beef serum) in the first stage of the reaction to insure complete activation of prothrombin.

Analysis for Thrombin. The quantitative procedure described by Seegers and Smith (3) was followed.

RESULTS

Platelet Accelerator. An accelerator of the first stage of coagulation was shown to be present in platelets by the same procedure used for demonstrating the presence of serum Ac-globulin in serum (9). When a dilute solution of purified prothrombin, standardized to contain 1.34 u/cc., is allowed to react with thromboplastin and calcium ions in the two-stage prothrombin procedure, the production of thrombin is quite slow and its eventual yield is comparatively low. If serum Ac-globulin is first added to the reaction mixture, the rate of thrombin production is increased in proportion to the quantity of Ac-globulin added and the yield of thrombin also increases. By increasing the Ac-globulin concentration stepwise, one obtains a series of prothrombin activation curves like those depicted on figure 1 (heavy solid lines). If plasma is used as a source of Ac-globulin one obtains a similar series of activation curves (10) but the shape of the curves is of a more rectangular nature (fig. 1, broken lines). The fundamental reason for the difference between plasma Ac-globulin and serum Ac-globulin resides in the fact that plasma Ac-globulin is the precursor of serum Ac-globulin (9) and must first be acted upon by thrombin in order to become the active accelerator.

When platelet extracts are examined by this procedure it becomes apparent that the extracts contain an accelerator of the first reaction of clotting. Furthermore it is present in an active form similar in that respect to serum Ac-globulin. In fact, the platelet extract can serve as a substitute for serum Ac-globulin, the kinetics of prothrombin activation being identical with either substance. This can be seen by

examination of figure 1 where two curves obtained with platelet accelerator show the same contours as those obtained with serum Ac-globulin.   If a given solution of platelet accelerator is matched in accelerator activity with a solution of serum Ac-globulin both can be diluted serially and the accelerator activity in each will remain quantitatively identical.   The serum Ac-globulin activation curves can, therefore,
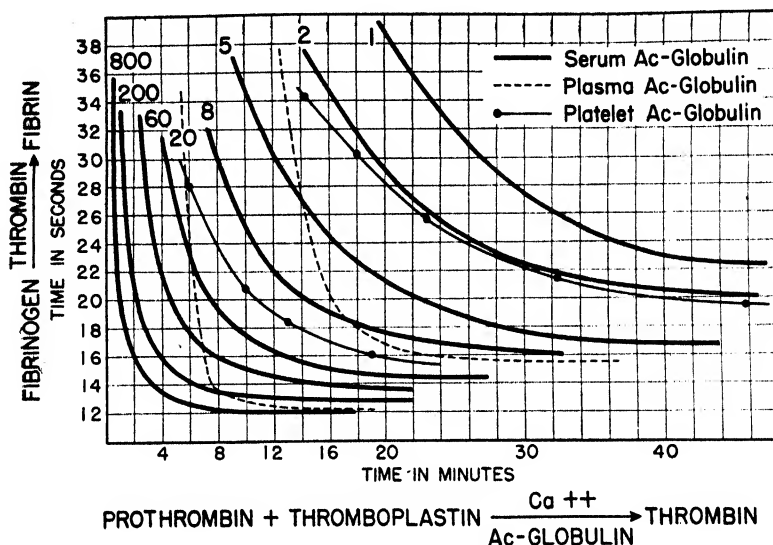
Fig. 1. ACCELERATION OF THROMBIN FORMATION by varying concentrations of serum Ac-globulin, plasma Ac-globulin, and platelet accelerator.   The heavy continuous lines represent prothrombin activation curves obtained by incubating graded amounts of serum Ac-globulin with constant amounts of purified prothrombin (1.34 u/cc.), thromboplastin, and calcium ions for varying lengths of time (abscissa) under the conditions specified by the 2-stage procedure for prothrombin analysis (5).   The thrombin formed in this reaction was measured by adding fibrinogen and recording the clotting time (ordinate).   The prothrombin and thromboplastin were essentially free of accelerator. Concentrations of serum Ac-globulin (u/cc. $\times$ 1000) are represented by the large numbers at the tops of the curves.

The two broken lines represent prothrombin activation curves obtained by the same method of analysis but with two different concentrations of plasma Ac-globulin instead of serum Ac-globulin. The slopes of these curves are not the same as the curves for serum Ac-globulin because the inactive plasma Ac-globulin is being changed to active serum Ac-globulin during the period of incubation. The two light continuous lines represent prothrombin activation curves obtained by the same method of analysis but with two different concentrations of platelet accelerator.   The slopes of these curves are identical with those for serum Ac-globulin.   Both platelet and serum accelerators are active and are not proenzymes like plasma Ac-globulin.

be used for the quantitative determination of platelet accelerator activity.   The assay procedure has accordingly been made identical to that already described for the quantitative determination of serum Ac-globulin (9), and, by definition, a unit of platelet accelerator is equivalent to a unit of serum Ac-globulin.

Relative Amounts of Accelerator in Platelets and in Plasma.   A comparison of the activation of prothrombin in the presence of serum Ac-globulin and in the presence of platelet accelerator reveals that approximately one-twentieth of the total accelerator activity derived from bovine blood is from platelts.   This comparison is

based on the assumptions that 100 cc. of bovine blood contains 0.5 cc. of centrifuge-packed platelets, and that the platelet accelerator is quantitatively extracted by the procedure outlined.

Properties of Platelet Accelerator. The physical and chemical properties of the accelerator present in the platelet extracts were investigated further. When heated at 53°C. for 10 minutes, 30 per cent of the activity was lost; when heated for 30 minutes, 87 per cent of the activity was lost. The accelerator remained fully active when the extracts were dialyzed against 0.9 per cent saline at room temperature. When a platelet extract was half saturated with ammonium sulfate, a white precipitate was formed. This precipitate, after centrifugation, solution in saline and dialysis to remove ammonium sulfate, contained 55 per cent of the original activity. This precipitate gave positive Biuret and Millon's tests. An aqueous solution of this precipitate was not coagulated by heat. From 70 to 80 per cent of the activity

TABLE 1. EFFECT OF PLATELET EXTRACT ON CLOTTING TIME OF A MIXTURE OF PURIFIED THROMBIN AND FIBRINOGEN

PLATELET EXTRACT	SALINE	THROMBIN SOLUTION	BUFFERED SALINE[1]	1 % FIBRINOGEN	CLOTTING TIME
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*sec.*
0	1	1	2	1	15.8
1	1	0	2		(clots in 5–10 min.)
1	0	1	2	1	11.0
0.5	0.5	1	2	1	12.7
0.25	0.75	1	2	1	14.1
0.125	0.875	1	2	1	15.1
0	1.5	0.5	2	1	30.0
1	0.5	0.5	2	1	19.7

All solutions were made in 0.9% sodium chloride. Temperature − 28°C.

[1] Made up so that the final clotting mixtures contained 1/10 its volume of imidazole buffer (11) and 0.15% calcium chloride (0.0136 M).

was sedimented by centrifugation for 45 minutes in a multispeed attachment rotor. The force was about 32,000 G.

Platelet Factor 2. This factor decreases the time required for thrombin to clot fibrinogen. Although this factor shows only moderate activity, failure to recognize its existence has probably resulted in faulty interpretations of experimental results.

The addition of a certain thrombin solution to purified fibrinogen caused a clot to form in 15.8 seconds (table 1). The addition of the same amount of thrombin plus platelet extract caused a clot to form in 11 seconds, even though the platelet extract alone did not clot the fibrinogen in 10 minutes. When a less concentrated thrombin solution, which caused clotting in 30 seconds, acted in the presence of platelet extract, the clotting time was reduced to 19.7 seconds. As an incidental observation it has been noted repeatedly that a mixture of thrombin and platelet extract produces a coagulum in about one minute. This coagulum is probably not fibrin even though it has much the same physical appearance. The coagulum can be

removed and the factor 2 effect can still be observed.   Factor 2 can also be demonstrated with the use of oxalated bovine plasma (table 2).

Properties of Platelet Factor 2.   Platelet extracts, prepared as described above, remained fully active when heated for 30 minutes at 53°C.   All the activity also remained when the extract was dialyzed against saline for one hour.   By half saturating an extract with ammonium sulfate, about half the activity could be demonstrated in the dissolved precipitate, after the latter solution had first been freed of ammonium sulfate by dialysis.   All the activity remained in the supernatant solution after centrifugation at 32,000 G. for 45 minutes.

Thromboplastin in Platelet Extracts.   In order to obtain an estimate of the thromboplastic activity of platelet extracts they were allowed to react with purified prothrombin both in the presence and in the absence of Ac-globulin.   The pH was adjusted to 7.2 by addition of imidazole buffer (11).   Calcium chloride was supplied at a concentration of 0.15 per cent (0.0136 M) in the final mixtures.   When platelet extracts were incubated at 28°C. with sufficient prothrombin to give a final concen-

TABLE 2. EFFECT OF PLATELET EXTRACT ON CLOTTING TIME OF A MIXTURE OF PURIFIED THROMBIN AND OXALATED BOVINE PLASMA

PLATELET EXTRACT[1]	OXALATED BOVINE PLASMA	SALINE	THROMBIN[1]	CLOTTING TIME
				sec.
o	2 parts	1 part	1 part	25
1 part	2 parts	o	1 part	1.76
o	2 parts	$1\frac{2}{3}$ part	$\frac{1}{3}$ part	105
1 part	2 parts	$\frac{2}{3}$ part	$\frac{1}{3}$ part	45
1 part	2 parts	1 part	o	no clot

[1] Made up in 0.9% sodium chloride solutions.

tration of 1.34 U/cc., no detectable amount of thrombin was formed in periods up to one hour.   Neither did the addition of purified serum Ac-globulin to the mixture of prothrombin and platelet extract result in thrombin formation in a one-hour period.   Addition of tissue thromboplastin to the mixtures of prothrombin and platelet extracts caused a rapid production and maximum yield of thrombin in three to five minutes.

When the prothrombin concentration was increased to 330 U/cc., approximately physiological concentration, the addition of platelet extracts caused a slow but definite production of thrombin.   A 20 per cent yield of thrombin developed in a period of 2 hours and after 24 hours the titer was not much higher.   These experiments indicate that the platelet extracts contain only a small amount of thromboplastin, if any.   The experiments do not indicate whether the platelet accelerator can activate some prothrombin.   This possibility seems remote.

DISCUSSION

In agreement with recent reports of others (1, 2) which were based on clotting times of mixtures of plasmas taken from various individuals, our results obtained

with purified prothrombin indicate that platelets contain only a small amount of thromboplastin. The relatively large amount of accelerator present in platelets was probably mistaken by early investigators for thromboplastin. Feissly (12) mentions the presence of two thromboplastic substances in platelets, one of which is heat stable and the other heat labile. His thermolabile platelet thromboplastin is probably the platelet accelerator described in this paper and should properly be regarded as an accelerator rather than as thromboplastin.

Platelet accelerator and serum Ac-globulin (9) appear to be similar with respect to their acceleration of prothrombin activation, their precipitation by half saturation with ammonium sulfate, and their destruction by heating at 53°C. The two accelerators differ somewhat, however, in their thermo-stabilities at 53°C. (table 3), serum Ac-globulin being destroyed more rapidly. A marked difference between the two substances lies in the fact that platelet accelerator is mostly sedimented at high centrifugal speeds, whereas serum Ac-globulin, in the form of bovine serum, is not sedimented under these conditions. Therefore, it appears that the two accelerators are entirely different proteins.

TABLE 3. STABILITY OF PLATELET EXTRACTS AND OF SERUM AC-GLOBULIN AT 53°C.

TIME	Platelet Extracts		Serum Ac-Globulin
	Factor 1	Factor 2	
min.	%	%	%
0	100	100	100
10	70	100	
15			7
30	13	100	0

Platelet factor 2 decreases the time of clot formation when present in mixtures of thrombin and fibrinogen. This property of platelet extracts diminishes readily on dilution so that it could not be expected to affect the rates of clot formation in highly diluted plasmas. However, where the plasma is used in high concentrations it is feasible that enough platelet factor 2 could be present to decrease clotting times appreciably.

Platelet factor 2 may be associated in some way with the substance in these platelet extracts which clots in the presence of thrombin. This substance is very similar in appearance to fibrin. However, its precursor is probably not fibrinogen because it appears to be unaffected by heating at 53°C. for 30 minutes. In addition, it is not coagulated and remains clottable with thrombin even after being heated. Fibrinogen is completely denatured under these conditions. It remains to be seen whether these ideas can be developed.

Before attempting to integrate the information on platelet extracts with recent observations on prothrombin activation it is necessary to review certain pertinent facts. *a*) It has been shown that thrombin can be derived from purified prothrombin even in the absence of thromboplastin, Ac-globulin or calcium ions (4). This indicates that prothrombin, by itself, contains all the necessary structural material for the formation of the thrombin molecule, and further that thromboplastin, Ac-globulin and calcium ions act merely as catalysts of thrombin formation. It appears

that the only way to escape this conclusion is to postulate that the thrombin formed in the presence of thromboplastin, Ac-globulin, and calcium ions is different from that derived from prothrombin in their absnce. *b*) Thromboplastin and calcium ions, in the absence of Ac-globulin, activate purified prothrombin slowly with a loss in total yield of thrombin (4). *c*) Plasma Ac-globulin is activated by small amounts of thrombin (9).

The experiments reported in this paper demonstrate that platelets contain an active accelerator and also small amounts of thromboplastin. These platelet constituents appear to be present in sufficient amounts to catalyze the formation of enough thrombin to change the inactive Ac-globulin of the plasma to the active form. In this way the platelets appear to retain their traditional rôle of trigger mechanism. This was recently again demonstrated very convincingly by the work of Brinkhous (13). More data are required for the rigid formulation of the exact rôle of thromboplastin of platelet origin and of the relative effects of the various amounts of prothrombin, Ac-globulin, calcium and thromboplastin on thrombin formation. Probably, under physiological conditions, thromboplastin of tissue origin is the chief source of this substance.

Although a number of uncertainties remain, it nevertheless seems appropriate to attempt to harmonize known facts as follows: thromboplastin of platelet origin, tissue origin, or both, according to the circumstances of the wound or the drawing of the blood, acts on plasma prothrombin to form a small amount of thrombin; platelet accelerator serves to catalyze the initial stages of this reaction; plasma Ac-globulin is activated by the first small amount of thrombin to serum Ac-globulin. The latter then becomes the main accelerator; and, finally, thrombin is produced rapidly and a clot forms. This correlation of events in clotting is in harmony with those made previously (9, 14, 15) on the basis of plasma constituents alone. The equations may be represented as follows:

$$\text{Thromboplastin} + \text{Prothrombin} \xrightarrow[\text{Platelet Accelerator}]{Ca^{++}} \text{Thrombin}$$

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin}$$

$$\text{Thromboplastin} + \text{Prothrombin} \xrightarrow[\substack{\text{Platelet Accelerator} \\ \text{Serum Ac-globulin}}]{C^{++}} \text{Thrombin}$$

$$\text{Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin}$$
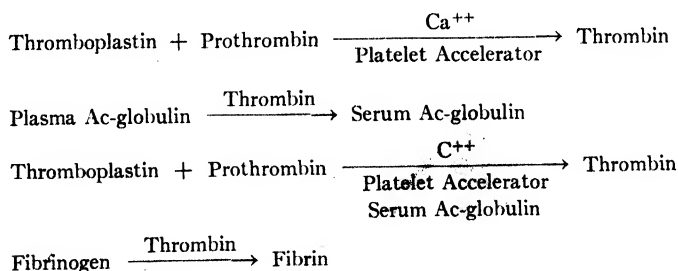
As the minor source of accelerator, platelets serve the primary function of beginning the events of clotting. Serum Ac-globulin furnishes the 'follow through'. A deficiency of either can thus cause a bleeding diathesis but neither of the two substances is absolutely necessary for thrombin production.

It has been pointed out previously that no one-stage method for prothrombin analysis can distinguish between the concentration of prothrombin and its activation rate (8, 16). The one-stage methods also cannot account for variations due to fibrinogen reactivity (17) and for variables due to the plasma medium itself from one species to another (3). Two further variables must now be considered; namely,

a) platelet accelerator and *b*) platelet factor 2, both of which may affect the rates of clot formation.

SUMMARY

With the use of purified clotting agents it has been shown that bovine platelet extracts contain an accelerator of prothrombin activation. They contain only a small amount of thromboplastin. The accelerator is apparently present in platelets in the active form and acts in a similar manner to serum Ac-globulin. The platelet accelerator is apparently a protein. It is destroyed by heating at 53°C., is non-dialyzable and is precipitated by half saturation with ammonium sulfate. It is sharply distinguished from Ac-globulin by being mostly sedimented by centrifugation at 32,000 G.

Bovine platelets also contain a substance which hastens the second stage of clotting. This substance is non-dialyzable, stable to heat at 53°C. for 30 minutes, precipitated by half-saturation with ammonium sulfate, and is not sedimentable by centrifugation at 32,000 G. Platelet extracts shorten considerably the clotting time of bovine plasma.

It has been postulated that platelets aid in the initial formation of thrombin primarily by catalyzing the interaction of prothrombin and thromboplastin. This thrombin then activates the inert plasma Ac-globulin to its active counterpart, serum Ac-globulin, which acts as the principal accelerator of the first stage of clotting.

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ABILITY OF THE STOMACH TO PRODUCE ELECTRICAL ENERGY

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A N ATTEMPT has been made in previously published work to throw further light on the intimate mechanism of gastric secretion by studies on the electrophysiology of the stomach. Specifically, the experiments were designed to test the hypothesis that electrical energy provided the energy necessary for the production of the osmotic work involved in the formation of the HCl of gastric secretion. Implicit in this hypothesis is the assumption that the mucosa of the stomach can produce sufficient electrical energy for the required amount of osmotic work.

In a former paper (1) an attempt was made to determine the ability of the stomach to produce electrical energy by placing low resistance, non-polarizable electrodes on opposite sides of the stomach wall and shunting these through a low external resistance. These findings can be illustrated by reference to the equivalent circuit of figure 1A where E_s represents the electromotive force of the stomach, R_s the resistance of the stomach wall, R_e the resistance of the electrodes applied to opposite sides of the stomach wall, R_x a fixed resistance of one ohm, and S a switch for closing the circuit. The potential difference (P.D.) was measured across the stomach wall with a potentiometer with switch S open. This switch was then closed and the electrical current in the external circuit was determined by measuring the IR drop across the one-ohm resistor. It was found that the current in the external circuit remained relatively constant with respect to time. Evidence was presented indicating that the total IR drop through the circuit was essentially equal to the open circuit P.D. across the stomach, and that the electromotive force, therefore, did not decrease with time. The average electrical energy production, under these conditions, was found to be 8.9 microwatts per cm.², which is only a small fraction of the amount of energy needed for the production of the HCl of the gastric secretion. It was pointed out, however, that this method probably does not measure the total ability of the electromotive forces of the stomach to produce electrical energy and that the total production of electrical energy might be much greater than that found with this method. For example, if there are shunts inside the stomach, this method would underestimate the stomach's ability to produce electrical energy. As will be seen in the following analysis, the problem of determining how much electrical energy the stomach can produce is an extraordinarily difficult one. There is one approach, however, that might be expected to throw further light on the problem. This approach is based on the finding that the magnitude of the electromotive force giving rise to the P.D. across the stomach wall apparently does not decrease with time during the flow of current under the conditions in the above described experiments. Since the electromotive force does not apparently decrease with time, it is reasonable to believe that it could produce more electrical energy than the amounts obtained in the above experiments. The question arises as to how much more electrical energy the electromotive force of the stomach, giving rise to the P.D. across the stomach wall, can produce. The present paper is primarily concerned with an attempt to obtain an answer to this question.

The essential principle of the method used in the present paper can be illustrated by reference to the circuit in figure 1B. This circuit is essentially the same as the circuit of figure 1A except that an external battery, E_x, is placed in series with the stomach. The electrical energy produced in the

circuit would be equal to $(E_x + E_s)I$ and that portion produced by the stomach equal to E_sI. The maximum ability of E_s to produce electrical energy (assuming no shunts in the stomach) could be determined by increasing the magnitude of E_x so as to increase the current I until the product of E_s and I reaches a maximum. The most difficult aspect of this problem is the determination of E_s during the passage of current across the stomach. It is conceivable that with this method the electrical energy production of E_s would be greater than the maximum electrical energy that could be obtained from E_s, if it were possible to connect a shunt of very low resistance directly to the 'terminals' of E_s. This possibility would depend on the relationship between the internal resistance of E_s and its ability to produce electrical energy when connected in series with an external electromotive force. It should be pointed out that the internal resistance of E_s is less than the total resistance across the stomach wall since it has been shown (13) that E_s originates somewhere between the submucosa and mucosal surface (probably nearer the mucosal than submucosal surface).

The magnitude of E_s could theoretically be determined during a period of current flow through the stomach, by measuring the P.D. across the stomach during this period. From the laws of electrical networks it follows that:

$$E_s = E_m - RI \tag{1}$$

where E_m is the P.D. measured across the stomach wall, R the resistance of the stomach wall, and I the magnitude of the current passing through the stomach. However, accurate calculations of E_s would depend on R remaining constant, and, since changes in E_m could be due to changes in R, this method would not yield reliable values for E_s.

The method used to determine E_s in the experiments described below was one in which, during a period of current flow, the circuit was momentarily broken at definite intervals and the P.D. across the stomach (open circuit voltage) measured with a string galvanometer. If the actual circuit of the stomach is that represented in figure 1A, i.e., a resistance and an electromotive force in series (assuming for the present that there is no electrical capacity of the stomach), the P.D. measured immediately after the break of the circuit would be equal to the electromotive force immediately before the circuit was broken. The electrical energy production by the stomach during current flow would then be equal to the product of this P.D. and the current in the external circuit during the flow of current. Since the actual circuit of the stomach is not known, the question arises as to whether the product of this P.D. and the current would be an accurate measure of the electrical energy production of the stomach if the actual circuit of the stomach was different from that assumed above. For example, if the actual circuit was one (fig. 1D) in which there was a shunt (R_2 of fig. 1D) across the electromotive force, would the above method be valid?

Application of the classical laws of electrical networks to the circuit represented in figure 1D reveals that

$$W = \frac{E_1 R_2}{R_1 + R_2} I_x + \frac{E_1^2}{R_1 + R_2} \tag{2}$$

In this equation W represents the electrical energy production of E_1 in watts when E_1 is in volts, the resistances R_1 and R_2 in ohms, and the current in the external circuit I_x in amperes. It can easily be shown that the open circuit P.D. from S_2 to M_2 is
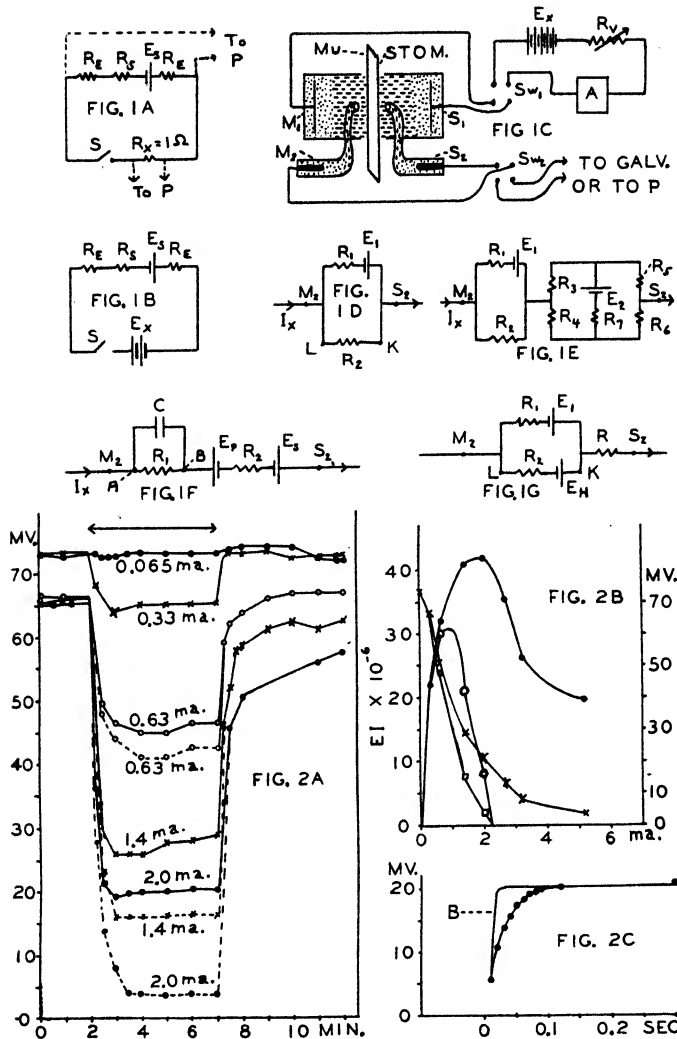
Fig. 1A. IN THIS CIRCUIT E_s represents the electromotive force of the stomach; R_s, the resistance across the stomach wall; R_E, the resistance of nonpolarizable isoelectric electrodes applied to the serosal and mucosal surfaces of the stomach; R_x, a resistance of one ohm; S, a switch and P a potentiometer.

Fig. 1B. E_X REPRESENTS A BATTERY. The other symbols have the same meaning as those of fig. 1A.

Fig. 1C. MU REPRESENTS THE MUCOSAL SIDE OF THE STOMACH, S_1 and M_1 electrodes placed opposite each other on the mucosal and serosal surfaces, S_2 and M_2 non-polarizable Zn-Zn acetate agar electrodes. The portions of the electrodes S_1 and M_1 in contact with the stomach were composed of 0.9% NaCl agar. Saturated KCl agar was present in electrodes S_2 and M_2 to make contact with the saline agar. Sw_1 and Sw_2 represent single throw, double pole switches; E_x, several lead storage batteries in series, R_v, a variable resistance; A, a milliammeter; *Galv.*, a string galvanometer and P, a potentiometer.

Figs. 1D and 1E. See text.

Fig. 1F. AN EQUIVALENT CIRCUIT OF THE STOMACH. C represents a static capacity in parallel with a resistance R_1, E_p a polarization electromotive force resulting from the flow of current, E_s the electromotive force of the stomach giving rise to the P.D. across the stomach wall in the resting condition.

equal to $\dfrac{E_1 R_2}{R_1 + R_2}$, and that $\dfrac{E_1^2}{R_1 + R_2}$ is equal to the electrical energy production by E_1 after the external circuit has been broken (I_x equals zero). It is true (as a corollary of Thévenin's theorem, see Bush, 2) that for any circuit (assuming no capacity nor inductance) that the total electrical energy output of the electromotive forces of the circuit, during the flow of an external current through the circuit, is equal to the product of the P.D. measured immediately after breaking the circuit and the current in the external circuit before the break of the circuit, plus the electrical energy production in the circuit immediately after the circuit is broken. Therefore, on the basis of the above considerations, the conclusion is warranted that the method used in the present work will not over-estimate the ability of the stomach to produce electrical energy, and will under-estimate it if there are shunts across the electromotive forces by an amount equal to the electrical energy production inside the stomach immediately after breaking the circuit.

An implication of the above analysis should be pointed out, and that is the possibility (and not an unlikely possibility considering the histology of the stomach) that there are electromotive forces oriented in such a way that their electrical energy output would not be influenced (or only to a small extent) by the electrical current sent through the stomach. A circuit illustrating this possibility is shown in figure 1E. In this circuit it is assumed that the resistances R_3, R_4, R_5, and R_6 are equal to each other and, therefore, the electromotive force E_2 would not contribute to the P.D. between S_2 and M_2. The passage of current through the circuit from an external battery would not change the electrical energy output of E_2. These conclusions can be easily verified by the application of the classical laws of networks to this circuit. Therefore, the method used in the present work could only be expected at best to determine the ability of those electromotive forces to produce electrical energy which are oriented in such a way as to contribute to the P.D. across the stomach.

For the sake of simplicity it was assumed in the foregoing analysis that the stomach did not possess an electrical capacity. Since the stomach undoubtedly does possess a capacity, the P.D. across the stomach following the break of the circuit would theoretically be a function of both the electromotive force and the discharge of the capacity. The electrical energy production, on the basis of the foregoing

Fig. 1G. See text.

Fig. 2A. EFFECT OF CURRENT FLOW ON P.D. ACROSS STOMACH. Period of current flow indicated at top of figure. Current density given in ma. per cm.[2] P.D. determined during period of current flow by momentarily breaking circuit and measuring P.D. with string galvanometer. Solid lines represent P.D. determined by ignoring rapidly rising phase in string galvanometer records. Broken lines represent P.D. determined by using initial values of galvanometer records.

Fig. 2B. ELECTRICAL ENERGY PRODUCTION of electromotive force of stomach in microwatts per cm.[2] vs. current density in milliamperes. Solid dots represent electrical energy production calculated from P.D. obtained by ignoring rapidly rising phase of galvanometer records. Open circles represent electrical energy output calculated from P.D. obtained by using initial values of galvanometer records. Crosses represent P.D. vs. current in which P.D. was determined by ignoring rapidly rising phase of records; squares represent P.D. vs. current in which P.D. represents initial readings of records.

Fig. 2C. P.D. vs. TIME, obtained from typical galvanometer record after interruption of current. Solid dots represent actual values obtained from the record; line through solid dots plotted from *equation 4* of text. Line B represents plot of *equation 4* in which R_1 was assumed to be equal to the total resistance of the stomach.

analysis, would be at least equal to the P.D. across the stomach, due to the electromotive force times the current.   In order to obtain the value for the P.D. due to the electromotive force, the measured P.D. would theoretically have to be corrected for the effect of the discharge of the capacity.   This can be illustrated by reference to the equivalent circuit shown in figure 1F, where C represents a static capacity. Immediately after the break of the circuit the P.D. between S_2 and M_2 would be equal to E_s (assume E_p in this circuit to be zero for the time being) minus the P.D. between points A and B due to the discharge of condenser C.   On the basis of measurements of the capacity of a wide variety of living tissues (3–6, 8) it has been found that the capacity of tissues in general is in the neighborhood of one microfarad per cm.^{2}.   Assuming the stomach possesses a capacity of approximately this magnitude and that R_1 of figure 1F is equal to the total resistance of the stomach, it can be shown (see formula below) that the P.D. between points A and B of figure 1F, due to the discharge of C, would decrease to less than one mv. within a few milliseconds.   The technique used in the following experiments was one in which the P.D. was measured with a string galvanometer at a relatively low camera speed so that the initial values of the galvanometer records would not be expected to be a function of the discharge of the capacity.   However, it was found that with relatively high current densities an initial rapidly rising phase of the P.D. was present in the galvanometer records. The possibility of this phase being due to the discharge of the capacity is discussed below.

<div align="center">METHODS</div>

A portion of the opened stomach of dogs anesthetized with sodium amytal (70 to 90 mg./kg. subcutaneously) was placed between two pairs of electrodes.   One pair of the electrodes S_1 and M_1 (fig. 1C) was connected in series with lead storage batteries E_z, a variable resistance R_v, a milliammeter A, and a double pole, single throw switch Sw_1.   The area of electrodes S_1 and M_1 in contact with the stomach was 11 cm.^{2}.   The other pair of electrodes S_2 and M_2 was connected to either a Hindle string galvanometer (without amplification) or a type K Leeds and Northrup potentiometer, via the double pole, single throw switch Sw_2.   The electrode S_1 was placed against the serosal surface and electrode M_1 was placed opposite electrode S_1 on the mucosal surface.   A variable resistance and a calibrating voltage (10 mv. steps, not shown in fig. 1C) were placed in the string galvanometer circuit.   The string galvanometer was calibrated before and after each experiment with switch Sw_2 closed and switch Sw_1 open.   Changes in the resistance of the circuit, comparable to the possible changes in the resistance of the stomach, produced no appreciable change in the galvanometer readings.   In the majority of the experiments a portion of the stomach wall was placed in a lucite chamber also containing two pairs of electrodes which will be referred to in the same way as those in figure 1C.   (See former paper, 9, for a detailed description of this chamber.)

The handles of switches Sw_1 and Sw_2 were fastened to a lever so that movement of the lever opened one of the switches and closed the other one.   In this way current flow through the stomach could be interrupted and the string galvanometer circuit connected to the electrodes S_2 and M_2.   In order to determine the total time elapsing

from the opening of switch Sw_1 to the first reading on the string galvanometer, both switches Sw_1 and Sw_2 were connected to the same voltage source and the lever connecting the switches was thrown and it was found that the break in the string galvanometer record was of about 5 milliseconds' duration.

The following experiments were performed on non-secreting stomachs unless otherwise specified.

<div align="center">RESULTS</div>

Figure 2 represents the results of typical experiments in which currents of various magnitudes were sent through the stomach for five-minute periods. The positive pole of the external battery (E_x of fig. 1C) was connected to the mucosal current sending electrode (M_1 of fig. 1C). The P.D. was measured before and after the current
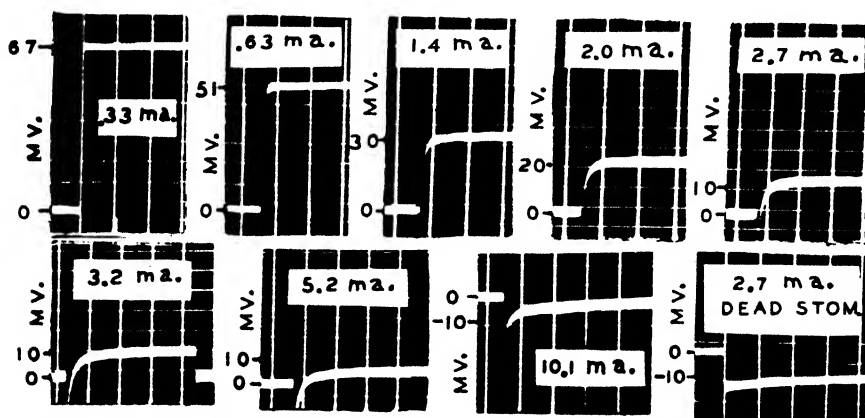
Fig. 3. STRING GALVANOMETER RECORDS OF P.D. across stomach following current flow of five minutes' duration. Numbers at top of each record represent current density in ma. per cm.² Time intervals between vertical lines equal to 0.20 second. Numbers at left of records represent P.D. in mv. Record in lower right corner represents the effect of current flow on a dead stomach.

sending period with the potentiometer. Current of a given magnitude was sent through the stomach and the circuit was broken at intervals, and the open circuit P.D. measured during these intervals with the string galvanometer. With the higher current densities (fig. 3) the string galvanometer record showed an initial rapidly rising phase. On the assumption that this rapidly rising phase is not due to a rapid change in E_s of figure 1F (or E_1 of fig. 1D), but to the decay of a back electromotive force (E_p of fig. 1F) or to the discharge of the capacity of the stomach, the P.D. used for the calculations of the electrical energy was determined by extrapolating the plateau portion of the curves to zero time (fig. 2C). In figure 2A the solid lines represent typical experiments in which the P.D. was determined in this way. It can be seen that with increasing current densities the magnitude of this P.D. is decreased. It can also be seen that for a given current density the value of this P.D. reached a relatively constant level within the first two minutes.

The P.D. for the calculation of the electrical energy output of the stomach was also determined on the assumption that the rapidly rising phase of the galvanometer

records is due to a rapid change in E_s (or E_1 of fig. 1C). With this assumption the
initial readings of the galvanometer records were used in calculating the electrical
energy output of the stomach. In figure 2A the dotted lines represent the change of
the magnitude of the P.D. determined in this way with time. It can be seen that the
magnitude of the P.D. determined in this way also reaches a relatively constant value
within approximately two minutes.

Figure 3 represents the string galvanometer records in a typical series of experiments after the current had been flowing for five-minute periods. The records obtained at the end of two, three, and four minutes were essentially the same as those
shown in the figure. It can be seen that with current densities of 0.63 ma. per cm.²
or less there was practically no rapidly rising phase of the curve, while a rapidly rising
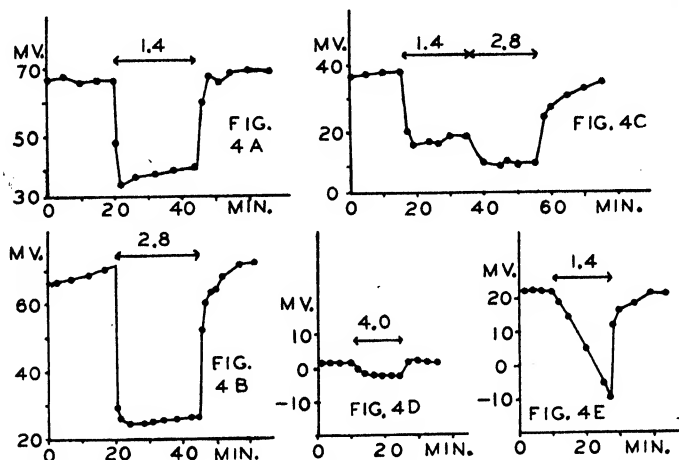
Fig. 4. EXPERIMENTS IN WHICH ELECTROMOTIVE FORCE WAS DETERMINED by momentarily
interrupting current and measuring P.D. across stomach with a potentiometer (see text). Period of
current flow indicated at top of each figure. Current density in ma. per cm.² Fig 4A and B represent experiments performed on non-secreting stomachs; fig. 4C, an experiment performed on a
histamine-stimulated secreting stomach; fig. 4D, an experiment performed on a dead stomach and
fig. 4E, an experiment on a dead stomach in contact with 0.16 N HCl.

phase was present with current densities of 1.4 ma. per cm.² and higher. It was also
found that the rapidly rising phase was absent in dead stomachs, as is illustrated in
this figure.

Following the rapidly rising phase the P.D. increases relatively slowly, and it
was found that at the end of two seconds following the break of the circuit the magnitude of the P.D. in the great majority of cases was not over 5 mv. higher than at the
end of the rapidly rising phase. It was found that readings could be obtained with
the type K potentiometer in about two seconds and that these values were essentially
equal to the magnitude of the P.D. obtained with the string galvanometer at the end
of two seconds.

A series of experiments were performed in which the P.D. following momentary
breaking of the circuit was measured in this way with the potentiometer. Typical
experiments in which this method was used are shown in figure 4. Figures 4A and
4B show experiments performed on non-secreting stomachs and figure 4C an experi-

ment performed on a (histamine-stimulated) secreting stomach. Figure 4D shows the effect of current flow on the P.D. of a dead stomach. A typical experiment is shown in figure 4E in which the ability of a potential originating at the junction of a 0.16 N HCl solution and a dead stomach to produce electrical energy is determined by this method. These experiments will be discussed more fully later on.

Calculation of the electrical energy output of the stomach. On the basis of the considerations discussed above, the electrical energy production of the stomach during the passage of a current through the stomach will be at least equal to the P.D., due to the inherent electromotive force following the break of the circuit times the current. Figure 2B represents a typical experiment in which the electrical energy output is plotted against the current density, using both of the above described methods for determining the P.D. (due to E_s of fig. 1B, or E_1 of fig. 1D). It can be seen in this experiment, using the method in which the rapidly rising phase of the P.D. is disregarded (referred to as the first method in the following), that the electrical energy output reaches a maximum at a current density of approximately 2.0 ma. per cm.[2]. Using this method, the average maximum electrical energy output in experiments performed on 6 dogs was 38 microwatts per cm.[2] with a range of values of from 29 to 44 microwatts.

Similar calculations were made in which the rapidly rising phase of the string record was not disregarded and the P.D. for electrical energy calculations was taken as the initial value of the P.D. on the string record (referred to as the second method in the following). As can be seen in the experiment in figure 2B, the maximum electrical energy production, using this method, was approximately three quarters of that determined by the first method. The actual average was 28 microwatts per cm.[2], with a range of values from 24 to 30. The magnitude of the current density at the point of maximum electrical energy output was in the neighborhood of 1.0 ma. per cm.[2] Because of the obvious sources of error in using the initial value of the P.D. of the galvanometer record in making these calculations, it should be pointed out that the above current density is near the level of current density at which the rapidly rising phase of the P.D. first makes its appearance.

In the experiments in which the P.D. was measured after the break of the circuit with the potentiometer, it was found that, using this P.D. in the calculations, the average electrical energy output was found to be 47 microwatts per cm.[2] (with a range of values from 40 to 78 microwatts per cm.[2]).

Analysis of the possible factors determining the magnitude of the P.D. following a period of current flow. Although not much is known about the actual factors responsible for electrical transients in living tissues (3, 5), it is possible for convenience of analysis to consider the rapidly rising phase of the P.D. following the break in the circuit to be a function of one or more of the following: *a*) the electrical capacity of the stomach (assuming a static capacity), *b*) changes in the electromotive forces giving rise to the resting P.D. across the stomach wall, *c*) the decay of a back electromotive force produced by the flow of current, and *d*) changes in the resistance of the stomach, i.e., changes in R_1 or R_2 if the circuit is similar to that of figure 1D.

It was assumed in the foregoing that the rapidly rising phase of the P.D. is not due to the discharge of the capacity of the stomach. The objection might be

raised, however, that these transients are of approximately the same duration as those found by Blinks (10) for Nitella and, therefore, might be considered as due to the discharge of a static capacity since Blinks' calculations gave a value of about one microfarad per cm.^{2} (11). However, calculations on this assumption give a relatively enormous value for the capacity of the stomach (the resistance of Nitella is of a higher order of magnitude than that of the stomach). A typical calculation illustrating this point is given in the following. The well known formula for the discharge of a condenser was used in the calculations (4, 12).

$$i = \frac{E}{R} e^{-(t/CR)} \qquad\qquad 3$$

During the flow of current I_x (fig. 1F) the condenser C of this equivalent circuit would be charged and the magnitude of the charge on the condenser would be equal to R_1I_x. Following the break of the circuit the condenser C would discharge through R_1, resulting in a P.D. between A and B of figure 1F in which A would be positive to B. This P.D. between A and B would, therefore, be oriented in the opposite direction to E_s, and the magnitude of this P.D. at a given instant would be equal to the value of E_s (extrapolated from the plateau portion of the curve, fig. 2C) minus the actual P.D. of the string record. With these considerations in mind *equation 4* can now be written:

$$V_{AB} = IR_1 = R_1I_x e^{-(t/CR_1)} \qquad\qquad 4$$

where V_{AB} is the P.D. between points A and B in volts and at any instant of time is equal to IR_1, where I is the current in amperes flowing through R_1. The initial charge E on the condenser C in volts is equal to R_1I_x, t is the time interval in seconds following the break of the circuit, and C the capacity in farads. In a typical experiment in which I_x was 2.0 ma. per cm.^{2} the values for V_{AB} in mv. at given time intervals were found to be: 14.5 mv. at 10 msec., 10.0 mv. at 20 msec., 6.5 mv. at 30 msec., and 2.9 mv. at 50 msec. It was found by substituting these values in *equation 4* and solving these equations, that C is equal to 2260 microfarads per cm.^{2} and R_1, 10.9 ohms per cm.^{2}. The total resistance between electrodes S_2 and M_2 was 382 ohms per cm.^{2}. This resistance was determined before the above experiment was performed by measuring the P.D before and during the passage of a very small current (0.03 ma. per cm.^{2}) through the stomach and calculating the resistance from *equation 1* (assuming E_s and R_s are unchanged by this current). The resistance from S_2 to M_2, measured without the stomach, was 137 ohms per cm.^{2}. Hence the resistance of the stomach ($R_1 + R_2$ of fig. 1F) would be 245 ohms per cm.^{2}.

Substitution of the values for C and R_1 obtained above in *equation 4*, yields an equation that gives an exceptionally good fit to the P.D.-time curve up to the plateau portion of the curve. It should be pointed out that, if an arbitrary value of R_1 is used such as the total resistance of the stomach (5, p. 26) and the first pair of values ($t = 10$ msec. and $V_{AB} = 14.5$ mv.) substituted in the equation, the value of C is lower than that obtained above but the resulting equation fits the actual curve very poorly (curve B of fig. 2C). The values for C, calculated from the other experiments, reveals that the values are all of the same order of magnitude as that given in the ex-

ample above. It might be concluded, therefore, because of the relatively large calculated value of C that the rapidly rising phase of the P.D. is not due to the discharge of a static capacity, but to one or more of the other factors enumerated above. However, if the effective surface of the stomach is considered as including the total area of the crypts and tubules, then the capacity of a cm.² of this surface would be much less than that calculated above and R_1 would be much larger, so that it is possible that the rapidly rising phase may be considered as due to the discharge of the capacity. It should be pointed out that the rapidly rising phase of the P.D. is not present in the dead stomach (fig. 3), but is still present in the intact stomach after the flow of large currents has reduced the P.D. to very low values (fig. 3).

Assuming that the P.D. following the break of the circuit is due to the sum of a polarization potential E_p of figure 1F and E_s, the electrical energy output of the stomach would be equal to $(E_s - E_p)I_x$. Actually the main interest of the authors is in the ability of E_s to produce electrical energy and, therefore, the P.D. used to calculate the electrical energy output of E_s would be equal to the P.D. of the galvanometer record plus the value of E_p. It should be mentioned that E_p could conceivably contribute to the P.D. across the stomach for a longer period than the duration of the rapidly rising phase of the P.D. For example, it is possible that the value of the P.D. measured five seconds following the break of the circuit is a more accurate measure of E_s than either of the above methods. If this value is used in calculating the electrical energy output, then the output of E_s was found in some experiments to be over 120 microwatts per cm.².

It is not possible on the basis of the present data to determine rigorously the contributions of the various factors given above to the actual P.D.-time curves. Although there would be many difficulties, it is possible that further experimentation might throw more light on the actual factors responsible for the P.D.-time curves. One obvious and not too difficult approach would be to determine whether or not the flow of current results in the production of a P.D. across the outer muscle layers of the stomach. If it did, this P.D. could not be due to changes in the electromotive force giving rise to the resting P.D. of the stomach, since it has been shown (13) that this electromotive force originates somewhere between the submucosa and mucosal surfaces.

DISCUSSION

Comparison of the electrical energy output with the minimum free energy necessary for the production of HCl. Comparison of these two characteristics reveals that the electrical energy output of the resting stomach determined by the above methods is definitely less than the energy needed for the production of HCl. For example, in a typical experiment the maximum electrical energy output of the resting stomach (determined by the first of the above methods) was found to be 39 microwatts per cm.². Following histamine stimulation this same stomach secreted HCl at a maximum rate of 0.013 mg. per cm.² per minute. Previous calculations (9) have shown that the minimum free energy needed for the production of 1 mg. of HCl is equal to approximately 1.1 joules, so that the minimum free energy needed to produce 0.013 mg. of HCl would be approximately 0.014 joule. Converting this latter value to

microwatts, it is found that the minimum rate of production of free energy needed to produce HCl is 233 microwatts per cm.[2]. Therefore, in this experiment the electrical energy production determined by the first of the above methods is found to be only about one sixth of the minimum amount needed for HCl production. Using the second method for determining the electrical energy production, the amount of electrical energy produced would be only about one ninth of the minimum amount needed for HCl production. Two similar experiments were performed with essentially the same results. The rate of HCl secretion found in these experiments was less for unknown reasons than the average rate of secretion found in previously published experiments (7, 9, 14). If the average rate of secretion of HCl from previous experiments is compared to the average rate of production of electrical energy of the present experiments, it is found that the electrical energy production, instead of being one sixth or one ninth of the minimum free energy needed for the production of HCl, is closer to one fourteenth or one twentieth.

In the above experiments the electrical energy production was determined on non-secreting stomachs. It is possible that the electrical energy output of the secreting stomach might be greater than that of the resting stomach. A few experiments were performed on secreting stomachs (3 dogs) in which the P.D. following the break of the circuit was measured with the potentiometer. It was found with this method (fig. 4C) that the electrical energy output of the secreting stomach was not markedly different from that found by the same method for the resting stomach.

Before the conclusion is warranted that there is not enough electrical energy available for the production of HCl, it would have to be shown that the methods used in the present experiments are adequate methods for the determination of the total electrical energy production of the stomach. As pointed out above, the methods used in the present work will at best only determine the ability of those electromotive forces to produce electrical energy that are oriented in such a way as to contribute to the P.D. across the stomach. If there are locally completed circuits within the stomach, as illustrated in figure 1E, then the amount of electrical energy produced would be greater than that determined by the present methods. Furthermore, if there are electromotive forces oriented so that they do not contribute to the P.D. across the stomach, the present method would not determine the ability of these electromotive forces to produce electrical energy.

It is conceivable that there are electromotive forces oriented across the cells of the gastric tubules toward the lumen of these tubules which would not contribute (or would contribute only a small component of their electromotive force) to the P.D. across the stomach, and that the ability of these electromotive forces to produce electrical energy may be many times (because of the relatively large surface area ratio) the ability of those electromotive forces to produce electrical energy that are oriented so as to contribute to the P.D. across the stomach. Therefore, an answer to the question as to whether or not the stomach electromotive forces can produce enough electrical energy for the production of HCl will have to await further experimentation.

Analysis of the electrical energy theory of osmotic work. An implication of the theory that the stomach uses electrical energy for the production of HCl is that there

must be completed circuits inside the stomach, otherwise the stomach could only produce a very small fraction of the amount of electrical energy needed. The authors are unaware of any evidence in the literature demonstrating that, in tissues such as the stomach with a maintained P.D. across them, there are locally completed circuits (apart from the current that would flow between adjacent macroscopic areas of different P.D. through the fluid or tissues external to the mucosa of the stomach). In fact, attempts to test this important part of the hypothesis will probably be attended with great difficulties. The electrical energy theory of osmotic work would, therefore, have to postulate a circuit similar to the one shown in figure 1E. In previous work (9, 15) it has been shown that the flow of current from serosa to mucosa results in an increase in the production of HCl in the secreting stomach, and that the flow of current in the opposite direction results in a decrease in the production of HCl. With these facts in mind, it would be reasonable to postulate that the flow of current from K to L in figure 1D would result in the production of HCl in some part of the circuit between K and L. Since the flow of current through a resistance could not provide energy that can be used for useful work (9) it would have to be further postulated that there is a back electromotive force at the locus where electrical energy is transformed into osmotic work. The circuit in figure 1G is a circuit that would fulfill the above requirements. In this circuit E_H represents the locus where HCl is formed, and it is assumed that E_s is greater than E_H. A somewhat similar theory has been formulated by Crane, Davies, and Widdowson (16).

Apart from the problem of the production of HCl, there are some interesting implications of the present findings that will be discussed below.

Comparison of the electrical energy production of the stomach with that of the electric organ of electrophorus electricus. Nachmonsohn *et al.* (17) found, under their experimental conditions, that the electrical energy production of the electric organ is 67×10^{-6} joules per gm. of tissue per impulse. They found that the electric organ was fatigued when it discharged at a rate of 800 impulses per minute. The electrical energy output at this rate of discharge would be equal to 0.052 joules/gm/min. In a later paper (18) a higher rate of electrical energy production was found, i.e. 0.085 joules per gm. per minute (data from tables 1, 2, and 5 of their paper). The average electrical energy output of the mucosa of the stomach in comparable units was calculated and found to be 0.012 and 0.0088 joules per gm. of mucosa per minute by the two methods for determining electrical energy output. It can be seen that the electrical energy output of the stomach is approximately from one fourth to one tenth that of the electric organ. It should be kept in mind, however, that the values for the electric organ were obtained under conditions in which the electric organ was becoming fatigued, while the values for the electrical energy output of the stomach were obtained during a relatively steady state. It is possible that the maximum electrical energy output of the electric organ during a steady state might be much closer to the output of the stomach than the above figures would indicate.

Because of the high voltage produced by the electric organ it might seem unlikely at first glance that the electrical energy output of the electric organ is not markedly different from that of the stomach. However, it must be recalled that the total mass of the electric organ is relatively large and that the voltage of a cross section of the

organ weighing one gram would be a small fraction of the total voltage. Furthermore, a discharge of the electric organ lasts for only a few msecs. which, at the rate of discharge of 800 per minute, means that the current would be flowing for only a small fraction of the time, while in the case of the stomach the current is flowing continuously.

Maximum current density produced by the stomach. The present experiments indicate that the electromotive force giving rise to the P.D. across the stomach can produce a current density of approximately one ma. per cm.^{2} (or 3 ma. or more if the first method is used). This finding is of interest in the light of the recent work of Marsh (19) in which he demonstrated that the growth of neuroblasts *in vitro* can be controlled by the flow of electric currents. The threshold from this effect was in the neighborhood of 10 ma. per cm.^{2}. An implication of these findings is that the flow of electric current inside the developing organism might control the direction of growth of neuroblasts. The obvious objection to this hypothesis, that the electromotive force of tissues could not produce a continously maintained current of this magnitude, is somewhat negated by the findings in the present work. On the assumption that there are completed circuits inside the stomach, the density of the current flowing in a given direction, assuming uniform resistances, would be twice the current densities given above. Furthermore, if the resistances of the stomach were not uniform then the current density in certain regions might be much higher, i.e. as high as 10 ma. per cm.^{2} While the characteristics of the stomach may be quite different from those of embryonic tissues, the present findings indicate that the electromotive force of living tissues can produce maintained currents of relatively high magnitudes.

These findings also raise the interesting question as to the mechanism responsible for the production of the electromotive force. Most investigators have attempted to account for the maintained P.D. across tissues on the basis that the potential arises from unequal ion mobilities (20) or unequal ion solubilities (21). Lund (22), on the other hand, has suggested that these maintained P.D.'s are oxidation reduction potentials. An implication of Lund's theory is that cell membranes may act as first-class conductors (23, 24). The findings in the present work raise the question as to whether these various types of potentials can give rise to currents of the magnitude that the electromotive force of the stomach can produce. Obviously from our knowledge of oxidation-reduction cells (lead storage cells, etc.), it is evident that oxidation-reduction batteries can give rise to currents of much higher magnitudes than those produced by the stomach. The writers have not been able to find data in the literature on the maximum ability of electromotive forces, depending on unequal ion mobilities or solubilities, to produce electric currents. A preliminary attempt to obtain an idea of how much electrical current these latter potentials can produce was made by studying the effect of current flow on the potential produced by applying 0.16 N HCl to a dead stomach. A typical experiment is shown in figure 4E. The P.D. across the dead stomach, when 0.16 N HCl was applied to the mucosa, was found to be approximately 20 mv. It can be seen from figure 4E that with a current density of 1.4 ma. per cm.^{2} the P.D. rapidly declines to zero and becomes inverted. In other words, the electromotive force of this system (due undoubtedly to unequal ion mobilities or solubilities, or both) cannot give rise to the current densities pro-

duced by the living stomach. It might be argued that the decrease in the P.D. in this experiment was due to mixing of buffers from the dead stomach with the HCl solution, with a consequent reduction of its hydrogen ion concentration. That this was not the case is shown by the behavior of the P.D. following the period of current flow. It can be seen from figure 4E that the P.D. returned to approximately its original magnitude following this period. Also it was found that the pH of the HCl solution was not significantly changed following such an experiment.

There are many objections, however, against concluding that the stomach electromotive force must therefore be due to oxidation-reduction potentials. For example, it is possible that the effective surface of the living stomach may be much greater than that of the dead stomach, and therefore the density of the current flowing across the surface of the living stomach for a given current density in the external circuit may be much less than for a dead stomach. Also further work needs to be done on the ability of potentials due to different ion mobilities or solubilities to deliver current in which other species of ions and other interfaces are used. Nevertheless the present experiments offer a real challenge to those investigators who are interested in the mechanism of the production of the P.D.'s of living tissues.

SUMMARY

An attempt was made to measure the ability of the stomach to produce electrical energy by a method in which an external battery is connected in series with the stomach. It is shown that the electrical energy produced by the stomach under these conditions is at least equal to the product of the P.D. across the stomach due to the electromotive force following the break of the circuit and the magnitude of the current flowing through the stomach.

The P.D. due to the electromotive force was determined by momentarily breaking the circuit and measuring with a string galvanometer the P.D. across the stomach wall. With higher current densities a rapidly rising phase of the potential difference was found to be present in the galvanometer records. Calculations of the electrical energy production were made on the assumption that the rapidly rising phase of the P.D. was due to *a*) the decay of a back electromotive force produced by the flow of current and *b*) a rapid change in the inherent electromotive force of the stomach. In the first method the level of the P.D., following the rapidly rising phase, was used to calculate the electrical energy production by the inherent electromotive force of the stomach, and in the second method the initial reading of the P.D. was used. It was found by the use of these two methods that the average electrical energy production by the stomach was 38 and 28 microwatts per cm.², respectively. The possible factors responsible for the rapidly rising phase are discussed.

The electrical energy output of the stomach, with the above methods, was found to be definitely less than the minimum free energy necessary for the production of HCl. It was also shown, however, that the methods employed in the present work would underestimate the ability of the stomach to produce electrical energy if one or both of the following conditions are present: *a*) electromotive forces oriented in such a way as not to contribute to the P.D. across the stomach wall and *b*) locally completed circuits inside the stomach. It is concluded that it is not possible at pres-

ent to decide as to whether the stomach can produce enough electrical energy for the production of HCl.

The findings of the present work indicate that the electromotive force of the stomach can produce a continuous current of around 1 ma. per cm.^{2} (or current densities of several ma. per cm.^{2} on the basis of assumption a above). The implications of these findings are discussed. It is pointed out that these findings may throw light on the mechanism of the production of the electromotive force. It is shown that a potential produced by the application of 0.16 N HCl to a dead stomach (a potential undoubtedly depending on different ion mobilities or solubilities) cannot produce continuously maintained currents of the magnitude produced by the living stomach.

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SALT EXCRETION IN DESERT MAMMALS[1]

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THE excretion of water from the animal body is regulated so that the water content of the body is kept relatively constant. If the intake of water is restricted, the response is less excretion of water through the kidneys, or, in other words, the urine will be more concentrated with respect to the solid excretory products.

Krogh (1) says: "The organs of excretion are in the higher vertebrates developed to serve the function of conservation of water." Krogh discusses the salt concentrations found in vertebrate urine from the viewpoint of water conservation in animals with restricted water supply. He finds that the highest urine concentrations are observed in whales, which have a heavy physiological load because their only source of water is the food organisms. Recently it has been demonstrated (2, 3) that certain desert animals have a considerably more efficient excretory system.

In view of those results we wanted to test the excretory ability in these desert animals with respect to electrolytes and chlorides, by imposing a heavy load of sodium chloride through the diet.

The animals used were two species of the family Heteromyidae, the kangaroo rat (*Dipodomys merriami*) and the pocket mouse (*Perognathus baileyi*). These animals can live indefinitely without drinking water and gain weight on a diet of dry grain only. The extra load with sodium chloride was accomplished by feeding grain containing 10 per cent by weight of NaCl.

1000 gram rolled barley was soaked in 2.8 liters of a 10 per cent NaCl solution. After 24 hours 1.8 liters could be drained off, which means that 1000 ml. solution (100 gram NaCl) was left in the grain. Then the grain was dried at 105° C., leaving about 10 per cent NaCl by weight in the dry food.

EXPERIMENTAL RESULTS

We used five *Perognathus* for testing the maximum salt excretion in the urine. The animals had lived for five to six weeks on a diet of dry grain only and no water before the experiment was started. The four of them had gained considerable weight in this preliminary period. They were then transferred to the salt grain diet for two days, and subsequently to dry grain again. During the salt grain diet all animals lost weight (aver. 8 per cent), but it is amazing that after the severe strain of

the excessive salt intake the animals were able to gain weight again on a completely dry diet. In two days the average gain was nearly 3 per cent and they continued to increase in weight. Most of these animals were still alive six months later, and they were fat and healthy.

We do not know how much of the food was consumed by the animals. (Exact studies of food consumption are made fairly complicated in these animals because they store food in their cheekpockets.) However, a considerable amount of the grain was actually eaten, and as a consequence the urinary salt concentrations increased tremendously.

The figures in table 1 give the chloride concentrations in the urine the second day on the salt diet. All the animals had very high chloride concentrations and electrolyte concentrations in the urine. When the animals are on their natural diet chlorides ordinarily make up only a small fraction of the electrolytes in the urine. However, on the salt diet about three fourths of the electrolytes were chlorides.

TABLE 1. URINE CONCENTRATIONS IN POCKET MICE AFTER TWO DAYS OF EXCESSIVE SALT INTAKE

DAYS ON DRY GRAIN	WT. LOSS, % AFTER 2 DAYS ON SALT GRAIN	WT. GAIN, % AFTER 2 DAYS ON DRY GRAIN	URINE CONCENTRATIONS AFTER 2 DAYS ON SALT GRAIN DIET		
			Chloride	Electrolytes	Urea
			mN	*mN*	*mN*
35	7.7	3.9	762	1070	2610
35	8.2	2.4	—	—	—
34	8.3	1.9	845	1140	1730
43	8.9	3.7	758	980	1270
44	9.1	2.2	515	1160	1570
Av.: 38	8.4	2.8	720	1088	1795

The urea concentrations are considerable but do not reach excessively high values. All urea concentrations are higher than can be reached by man, and the highest value is a little higher than can be reached by the white rat. It is clear that the urea excretion to a considerable degree can be maintained simultaneously with high electrolyte excretion. A simultaneous load with salt and urea was never tried, but would surely give interesting results.

As mentioned above the animals recovered again on a diet of dry grain only, so the excessive salt load had no ill effects that could not be repaired. There is no doubt, however, that the animals are unable to survive on the salt grain diet.

The pocket mice lost considerable weight in two days and would not have been able to survive for a long period. In a group of kangaroo rats which was given the salt grain the longest surviving individual lived for 17 days (table 2).

Most of the kangaroo rats died after about a week, but even this length of survival must be considered a remarkable accomplishment. It might be expected that the animals, after an initial period, practically stopped eating and died from partial starvation. However, when completely starved the kangaroo rats live for only three days or less (seven individuals kept without food died after 1, 2, 2, 3, 3,

3, and 3 days, respectively).   We noticed, much to our surprise, that the animals
did not show any high degree of desiccation at the time of death (table 2).   Ordinarily

TABLE 2. SURVIVAL TIME OF KANGAROO RATS ON A DIET CONTAINING 10% NaCl

DAYS SURVIVAL ON SALT GRAIN DIET	WT. LOSS	WATER CONTENT AT DEATH; PERCENTAGE OF BODY WEIGHT
	%	
2	14.8	69.2
4	14.7	69.5
4	21.3	68.3
5	22.1	68.9
6	25.9	67.8
6	26.9	68.6
7	24.8	70.6
7	28.4	67.2
8	23.2	67.5
11	33.6	69.4
17	36.6	68.8
Av.: 7	24.8	68.7

TABLE 3. URINE AND PLASMA CONCENTRATIONS IN KANGAROO RATS FED GRAIN CONTAINING
10% NaCl

DAYS ON SALT GRAIN DIET	WEIGHT LOSS	WATER CONTENT	URINE			PLASMA		
			Chloride	Electrolytes	Urea	Chloride	Electrolytes	Urea
	%	%	mN	mN	mN	mN	mN	mN
2	10.9	68.4	507	625	810			11.3
	11.5	69.9	773	805		142	158	13.4
	15.5	69.7		808	3,120		161	11.8
	16.7	67.4	728					
	17.6	70.6	908	1,220	2,090		157	26.3
Av.:	14.4	69.2	729	865			159	15.7
6	11.3	68.0	651	1,000		121	154	
	15.2	69.2		1,010		122	158	
	16.4	68.5		537		111	153	
	19.1	69.0	586	776		123	162	
	20.8	67.7				155	163	
	26.6	68.8	229	1,000		135	162	21.5
Av.	18.2	68.5		865		128	159	

an ingestion of large amounts of salts causes heavy diarrhea and dehydration.
Diarrhea was not observed in these animals.

Excretion from the kidneys was much alike in the kangaroo rats and the pocket
mice.   We found some extremely high urinary concentrations (table 3; the table is
incomplete because of difficulties in obtaining urine samples large enough for all

analyses.)   The data include the highest chloride value ever found in urine, 908 mN (equal to 5.3 per cent NaCl).   In the same sample we have the highest concentration of electrolytes, 1220 mN, which is more than twice as concentrated as sea water. Adolph (4) gives the following maximum urinary concentrations of chloride: rat, 600 mN; man: 370 mN; dog: 330 mN; goat: 320 mN.

Irving *et al.* (5) were interested in the water balance of the seal.   Since the seal has no access to drinking water it has quite an interesting water problem.   It turned out that the seal can maintain the water balance with the water of the food (fish) because of the fairly high water content in the fish and the low evaporation from the seal itself.   As for the possibility of using sea water for drinking Irving and colleagues say "The kidney which could abstract water from a 3.5 per cent salt solution would be performing osmotic work at an amazing intensity."   This is truly so, and we can add that we were very much astonished to find an excretory system with twice that performance.

We have some samples from kangaroo rats which had lived for more than two days on the salt diet.   Also here we find some high urinary concentrations, but we do not have many simultaneous figures for urine and plasma.   We give a table of some figures obtained.   The plasma seems not to be excessively concentrated with respect to electrolytes, while the chloride values undoubtedly are above the usual level.

SUMMARY

Some desert rodents excrete a very concentrated urine, which enables them to expend only small amounts of water for excretion.   The maximum excretory ability with respect to electrolytes is about 1200 mN, and for chlorides about 900 mN. This appears to be far in excess of the limits known from other mammals.   This ability must be interpreted as a very useful mechanism for water conservation and an adaptation to desert life.

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EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CLEARANCE OF THE DALMATIAN DOG[1]

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IN A previous study (1), it was found that the administration of salicylate to rats produced an increase in their renal excretion of uric acid. Blood analyses and renal clearances done on these rats receiving salicylate, however, indicated that the observed increase in uric acid excretion was not preceded or accompanied by an increase in 1) blood uric acid, 2) glomerular filtration rate or 3) in renal plasma flow. These observations suggested that salicylate increased the excretion of uric acid by impeding the latter's renal tubular reabsorption. Talbott (2) previously had come to the same conclusion concerning the mechanism of action of salicylate in the human subject.

In view of the fact that recently we (3) discovered that the Dalmatian dog excreted uric acid at the level of glomerular filtration without subsequent tubular excretion or reabsorption, it seemed important to determine the effect of salicylate upon the excretion of uric acid in this species of dog. For if salicylate actually increases the output of uric acid in mammals by impedance of its tubular reabsorption, this drug should not be uricosuric in the Dalmatian dog, as the renal tubules of this animal normally do not reabsorb the uric acid present in the glomerular filtrate. The results of such a study are reported herein.

METHODS

Two healthy, male, thoroughbred Dalmatian dogs (litter mates) weighing approximately 20 kilos. each and 6 male mongrel dogs of approximately the same size were used in this study. Five uric acid and creatinine clearances were done on the 2 Dalmatians both before and during the intravenous injection of sodium salicylate. Eight similar combined clearances were done on the 6 mongrel dogs both before and during salicylate injection.

All dogs were anesthetized with pentobarbital sodium, catheterized and given a solution of 0.9 per cent Na_2SO_4 by vein at the rate of 4 cc. per minute until a urine flow of at least 1 cc. per minute occurred. The dogs then received an intravenous infusion of a solution containing 4 mg. of creatinine per cc. The animals received 100 cc. of this latter solution within 5 minutes, after which time they continued to receive it at the rate of 4 cc. per minute. After 30 minutes, the bladder was emptied, a blood sample taken and the first urine collection was begun. After 15 minutes, the bladder was emptied, a second blood sample taken and a second urine collection of 15 minutes was obtained in the same manner. After the second control urine collection had been taken, a solution containing 10 mg. of sodium salicylate, as well as 4 mg. of creatine per cc., was given by intravenous infusion at the rate of 4 cc. per minute for the remainder of the experiment. Each dog received approximately a total of 3.5 gm. of sodium salicylate. Thirty minutes after this last infusion had begun, a third and fourth urine collection (each of 15 minutes duration) with appropriate blood

samples were taken exactly as described in the control period. Determinations of the uric acid and creatinine in the blood and urine samples allowed the calculations of the uric acid and cretainine clearances.

Uric acid in plasma and urine was determined according to the method of Folin (4). Creatinine determinations were done according to the method of Folin and Wu (5).

TABLE 1. EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CLEARANCE OF THE DALMATIAN AND NON-DALMATIAN DOG

DOG	BEFORE SALICYLATE					AFTER SALICYLATE				
	UV[1]	PUA[2]	UAC[3]	CC[4]	UAC/CC	UV[1]	PUA[2]	UAC[3]	CC[4]	UAC/CC
Dalmatian dogs										
D1.........	4.7	0.38	99.0	99.0	1.0	4.5	0.40	87.2	88.0	0.99
D1.........	5.4	0.40	82.0	86.0	0.96	3.6	0.38	84.0	89.0	0.95
D1.........	3.7	0.47	73.0	66.0	1.10	2.5	0.54	71.0	62.0	0.82
D2.........	6.5	0.53	114.0	98.7	1.15	5.6	0.44	112.5	88.7	0.93
D2.........	7.0	0.43	108.5	112.5	1.05	4.0	0.65	95.8	91.0	1.05
Av........	5.4	0.44	95.2	92.4	1.05	4.0	0.48	90.1	83.7	0.95
Non-dalmatian dogs										
C1.........	8.1	0.22	28.9	86.0	0.32	7.2	0.17	48.4	83.0	0.58
C1.........	5.9	0.26	16.6	93.1	0.18	7.4	0.24	23.6	88.2	0.27
R2.........	7.5	0.22	31.7	104.0	0.30	4.3	0.23	33.5	105.0	0.32
R2.........	6.0	0.14	43.0	101.0	0.43	3.5	0.16	39.0	100.0	0.39
R3.........	7.1	0.23	21.9	95.0	0.23	4.4	0.23	42.5	83.0	0.51
N1.........	2.2	0.29	34.7	92.0	0.38	4.5	0.34	54.1	97.0	0.56
R4.........	3.6	0.18	30.0	98.0	0.31	3.4	0.21	37.5	107.0	0.35
S1.........	5.5	0.16	23.0	106.0	0.22	3.3	0.22	38.0	106.0	0.38
Av........	5.7	0.21	28.7	96.9	0.30	4.75	0.23	39.6	96.2	0.42

[1] Equals cc. of urine/min.   [2] Equals mg. of uric acid/100 cc. of plasma.   [3] Equals uric acid clearance in cc/min.   [4] Equals creatinine clearance in cc/min/sq. M. of S.A.

RESULTS

A. *Effect of Sodium Salicylate Upon 1) The Plasma Uric Acid, 2) Uric Acid Clearance and 3) Creatinine Clearance*

1) *Non-Dalmatian dogs.* As table 1 demonstrates, no significant change occurred in the plasma uric acid content of non-Dalmatian dogs after the infusion of salicylate. The average plasma content was 0.21 mg. per 100 cc. before, and 0.23 mg. during, the injection of salicylate.

The uric acid clearance, however (table 1), increased significantly after infusion of salicylate had been started. Thus the average uric acid clearance of the 6 non-Dalmatian dogs was 28.7 cc. per minute during the control period and 39.6 cc. per minute after the administration of sodium salicylate. Despite the decrease in urine volume (table 1) the creatinine clearance remained unchanged after the infusion of sodium salicylate had been started. Accordingly, the uric acid clearance/creatinine clearance changed from 0.30 (before salicylate) to 0.42 after salicylate had been given.

2) *Dalmatian dogs*. Similar to the findings in the non-Dalmatian dogs, the plasma uric acid content of the Dalmatian was not changed (table 1) by the infusion of sodium salicylate. Likewise, there was no significant change in the uric acid clearance after the administration of salicylate. Thus (table 1) the average uric acid clearance was 95.2 cc. per minute before and 90.1 cc. after the injection of salicylate.

Similarly the average creatinine clearance (92.4 cc. per minute) did not change significantly after injection of salicylate. As was observed previously (3) the average creatinine clearance of these dogs was approximately the same as their average uric acid clearance. The uric acid clearance/creatinine clearance ratio therefore remained approximately at unity (table 1) throughout the experiment. A rather marked decrease in the rate of excretion of urine however was noted after the salicylate infusion had been maintained for over 30 minutes. This latter decrease noted in both the Dalmatian and non-Dalmatian was thought to be due to the hypertonicity of the salicylate infusion.

DISCUSSION

In a previous study (1), uric acid was found to be increased in the urine of rats after the administration of sodium salicylate. It was thought that this uricosuric effect of salicylate was due to its probable ability to impede the reabsorption uric acid by the renal tubules. In a later study (3), however, the renal tubule of the Dalmatian dog peculiarly was found not to reabsorb or excrete uric acid, the latter substance being excreted at the level of glomerular filtration (i.e., equal to the excretion rate of creatinine). Therefore, if salicylate exerts its uricosuric effect by tubular interference in the reabsorption of uric acid, its action should not be uricosuric in the Dalmatian dog.

The observations of this present study indicate that whereas sodium salicylate has a clear uricosuric action in the ordinary mongrel dog, it has none in the Dalmatian dog. We believe this fact not only confirms our earlier observation concerning the uniqueness of the Dalmatian kidney with respect to excretion of uric acid, but also furnishes further evidence that the uricosuric effect of salicylate, when manifested, is due to the tubular actions of the drug.

CONCLUSIONS

The administration of sodium salicylate was not able to effect a change in the uric acid clearance of the Dalmatian dog. The significance of this observation in relation to the renal peculiarity of the Dalmatian dog and the mode of action of salicylate was discussed.

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MEASUREMENT OF RENAL FUNCTIONS IN RATS

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RECOGNITION of substances which stimulate growth of renal tissue and the activity of renal enzyme systems initiated the development of a method suitable for bio-assay of such materials from measurements of specific renal excretory functions in rats. The functions selected were the maximum tubular excretory capacity for p-aminohippurate (Tm_{PAH}) and the plasma creatinine clearance (C_{CR}). Tm_{PAH} is taken as measuring activity of tubular tissue and C_{CR} as equivalent to the rate of glomerular filtration.

Experience in the study of renal function in other species led to the premises that the method chosen should involve *a*) complete collection of urine during a state of rapid flow, while *b*) the plasma concentrations of PAH and creatinine during urine collection should be accurately estimated and *c*) the animal maintained under physiological conditions during the test.

Methods hitherto described (1–6) did not fulfill these criteria. The procedure selected is therefore described.

PROCEDURE

Apparatus. *a*) Rat holder (fig. 1); *b*) blood collection pipettes (fig. 1). These are drawn from soft glass tubing 4 mm. I.D.; *c*) ureteral catheter, no. 4 F, cut 6 inches in length; and *d*) box for heating at 45° C.

Solutions. *a*) Heparin in 0.9 per cent NaCl; 1 cc. contains 2 mg. heparin. *b*) PAH-mannitol-creatinine: sodium p-aminohippurate 6 gm.; mannitol 10 gm.; creatinine, 4 gm.; 0.9 per cent NaCl q.s. to 100 cc. *c*) Intracaine (Squibb) 2.5 per cent in 0.9 per cent NaCl.

Experimental. A female rat weighing 170 to 250 gm. was injected intraperitoneally with 0.3 cc. of heparin solution. The catheter was inserted into the bladder under light ether anesthesia. PAH-mannitol-creatinine solution was injected subcutaneously in two equal doses of 1.7 cc. per 100 gm. body weight. The time of this injection was noted as 0. The rat was then placed in the holder, adjusted so as to restrain movement (fig. 2). The position of the catheter was checked by observing urine flow. Discomfort from its presence was minimized by injecting 0.2 cc. of intracaine solution into the bladder, retaining it there for several minutes. Nearly all animals soon accustom themselves to the procedure and remain quiet during the period of observation. Those few which do not are rejected.

At 40 to 43 minutes after 0 time, rat and holder were warmed at 45°C. for 3 minutes in a box heated by an electric light bulb to this temperature. At 45 minutes the first sample of blood (B1) was collected by snipping the end of the tail and milking

out about 0.4 cc. of blood onto a heparinized watchglass. The blood was drawn into the pipette and the drawn-out end sealed in a flame. At 48 minutes the bladder was rinsed with four successive 0.2 to 0.5 cc. volumes of saline, the last rinse ending exactly at 50 minutes. These rinses were discarded. Urine collection for clearance measurement was begun at 50 minutes by directing the catheter into a small graduated cylinder. The bladder was rinsed in the same manner, beginning at 58 and ending exactly at 60 minutes. These rinsings were added to the urine and the mixture of
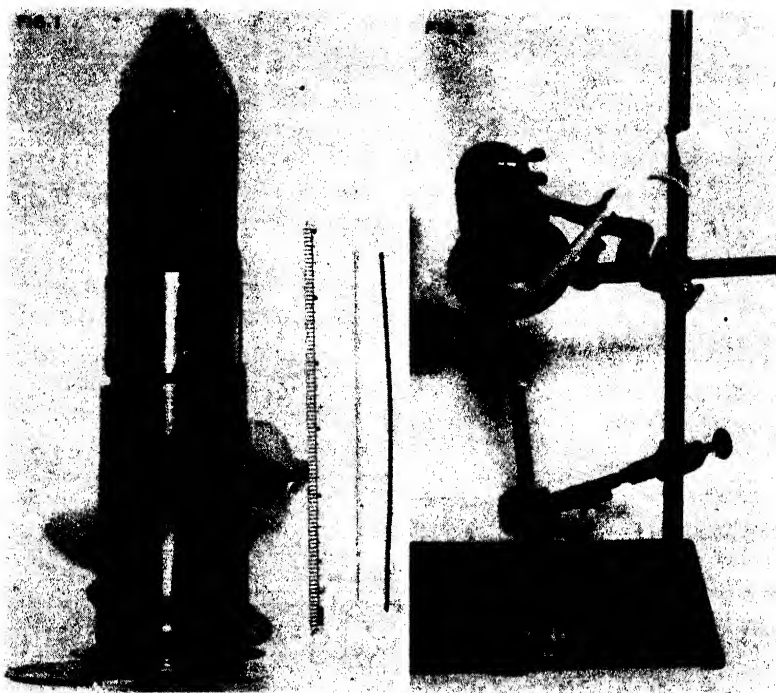
Fig. 1. RAT HOLDER CONSTRUCTION, blood pipette and catheter to scale
Fig. 2. RAT IN HOLDER during urine collection

urine and bladder rinse made up to 100 cc. in a volumetric flask. The rat was again warmed and blood collected (B2) at 65 minutes.

Blood samples were then centrifuged, the pipettes scored with a diamond point at the cell-plasma interface, the pipette broken and 0.1 cc. of clear plasma taken up in a volumetric pipette. A protein-free filtrate (1/100 dilution of plasma) was made by cadmium sulfate-sodium hydroxide precipitation. PAH was determined in filtrate and diluted urine by the method described by Goldring and Chasis (7) and creatinine by an adaptation of the method of the familiar alkaline picrate procedure. Mean plasma concentration during urine collection was found by interpolation on semi-logarithmic paper of the values found for B1 and B2.

RESULTS

Values obtained in rats of the Sprague-Dawley strain are presented in table 1, where they are arranged for comparison with estimates of the same and similar renal

functions made by others. The procedure described is at least as satisfactory as any. Reproducibility in successive estimates at intervals of 7 to 10 days in individual rats is shown in table 2. Values are reported per 100 gm. body weight rather than in

TABLE 1. SUMMARY OF OBSERVATIONS ON RENAL FUNCTIONS IN NORMAL RATS

SERIES	FUNCTION	NO. OF OBSER- VATIONS	ANIMALS	MEANS	σ MEAN	$\dfrac{100\sigma \text{ MEAN}}{\text{MEAN}}$
Own	C_{CR}	91	39	0.61	.016	2.6
	Tm_{PAH}		39	0.29	.0056	1.9
Braun-Menendez and Chiodi	C_{Inulin}	84	84	0.60	.031	5.2
	$Tm_{Diodrast-I}$	30	30	0.183	.0135	7.4
Friedman, M.	C_{CR}	31		0.66	.028	4.3
Friedman, S., Polley and Friedman	C_{Inulin}	14	14	0.65	.019	2.9
	Tm_{PAH}	14	14	0.18	.005	2.8
Dicker and Heller	C_{Inulin}	104	35	0.35	.0027	0.8
	$Tm_{Diodrast-I}$			0.126	.0027	2.1
Corcoran and Page	$C_{Mannitol}$	29	20	0.55	.085	9.7
	Tm_{PAH}	29	20	0.327	.016	4.9

Summary of observations of renal functions in this and other series of observations in normal rats. Data of other authors are recalculated, where necessary, applying corrections for body weight and urine volume to bring them in line with observations in the present series in which body weight averages 229 gm. and urine volume about 0.2–0.4 cc/10 min. Results are expressed as cc. plasma clearance or mg. Tm per 100 gm. body weight per minute. The data of Corcoran and Page were obtained by a method similar to that here described. The greater variability presumably reflects the effects of light anesthesia and inaccuracy due to single blood sampling.

TABLE 2. VARIABILITY OF RENAL FUNCTION DETERMINATIONS

RAT NO.	Tm_{PAH} MG/100 GM.							C_{CR} CC. PER 100 GM.						
	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
15	0.34	0.27	0.39	0.34			0.33	0.70	0.70	0.75	0.56			0.67
40	0.32	0.24	0.37	0.35	0.29	0.33	0.32	0.63	0.64	0.76	0.68	0.61	0.57	0.64
46	0.32	0.28	0.34	0.25			0.30	0.59	0.68	0.77	0.51			0.63
47	0.26	0.26	0.24	0.31			0.27	0.55	0.71	0.77	0.82			0.63
48	0.25	0.37	0.31	0.28	0.29		0.30	0.47	0.56	0.66	0.47	0.51		0.53
50	0.33	0.29	0.27	0.25			0.28	0.49	0.45	0.38	0.47			0.45
51	0.29	0.36	0.37	0.35			0.34	0.44	0.84	0.80	0.61			0.67
54	0.26	0.29	0.29	0.24			0.27	0.91	0.49	0.48	0.66			0.63
65	0.34	0.36	0.34	0.36			0.35	0.67	0.69	0.67	0.69			0.68
66	0.28	0.24	0.26	0.25			0.26	0.76	0.73	0.69	0.58			0.69

Successive observations of Tm_{PAH} and C_{CR} in normal rats at intervals of 7 to 10 days.

surface area units because of the simplicity of calculation and because, in the narrow range of body weight present in our group, the surface area units offered no advantage.

DISCUSSION

Factors making for accuracy in this method are *a*) complete collection of urine by bladder washing in contrast to digital expression of urine; *b*) the increased accu-

racy of collection due to the diuretic effect of mannitol (mean plasma mannitol concentration during the test period is about 150 mg/100 cc.); *c*) the volumes of blood withdrawn are small, so that the procedure is neither shocking nor depleting, while *d*) the analyses are done on plasma rather than whole blood which, in our hands, is not as satisfactory as plasma for determinations of PAH and mannitol and is unsuitable for creatinine; *e*) the brevity of the clearance period and the measurement of two blood samples minimize inaccuracies in the estimate of mean plasma concentration; *f*) the plasma concentrations obtained average about 50 mg. PAH and 25 mg. creatinine/100 cc.; analyses in normal rats indicate that the curve of plasma concentrations are either level or slowly falling; the concentrations are such as to be accurately measurable in 1/100 plasma filtrate.

The mean creatinine clearance found in our series corresponds well with other estimates of creatinine and inulin clearances. The mean Tm_{PAH} is greater than that reported by Friedman, Polley and Friedman (5). This is because the plasma concentration maintained in our procedure is such as to saturate the tubular excretory mechanism for PAH. That this is so is indirectly confirmed by the ratio of mean Tm_{PAH} in our series to mean $Tm_{DIODRAST-I}$ in the series of Braun-Menendez and Chiodi. The ratio is 1.58, which corresponds with this value in other species. The estimates of glomerular filtration and tubular excretory function respectively from inulin clearance and $Tm_{DIODRAST-I}$ by Dicker and Heller (2) are low in comparison with our observations and those of others. Insofar as these differences are not procedural, they may be attributed to differences between rat groups found by Corcoran and Page (6.)

SUMMARY

A method is described for the measurements of creatinine clearance and Tm_{PAH} in rats under conditions which favor accuracy and reproducibility. The procedure is adaptable to the bio-assay in rats of substances which affect these renal excretory functions.

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BUFFER EQUILIBRIA AND REABSORPTION IN THE PRODUCTION OF URINARY ACIDITY

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VARIOUS explanations for the titratable acidity of the urine have been reviewed by Pitts *et al.* (1–3), who postulate *active* transport of acid by the renal tubular cells, either as ionic exchange against a concentration gradient (4) or as frank acid secretion (5), as the mechanism responsible for titratable urinary acidity under all circumstances. They cite their experiments (1, 3) as conclusively demonstrating this mechanism.

The concentrations in glomerular filtrate of B_2CO_3 (about 10^{-4} M), BOH (10^{-7} to 10^{-6} M) and B_3PO_4 (10^{-8} M) are so extremely small that it has been generally held that these compounds can be of no physiological significance. However, since far larger amounts of B_2CO_3, BOH or B_3PO_4 could be reabsorbed from the glomerular filtrate than exist therein at any given moment, these compounds might play important rôles in the production of urinary acidity. So far as these compounds are concerned, the following equilibrium reactions must obtain:

$$BHCO_3 + B_2HPO_4 \rightleftharpoons B_2CO_3 + BH_2PO_4 \tag{A}$$
$$BHCO_3 + BCl + Creatinine\ (free) \rightleftharpoons B_2CO_3 + Creatinine \cdot HCl \tag{B}$$
$$HOH + B_2HPO_4 \rightleftharpoons BOH + BH_2PO_4 \tag{C}$$
$$HOH + BCl + Creatinine\ (free) \rightleftharpoons BOH + Creatinine \cdot HCl \tag{D}$$
$$2B_2HPO_4 \rightleftharpoons BH_2PO_4 + B_3PO_4 \tag{E}$$
$$B_2HPO_4 + BCl + Creatinine\ (free) \rightleftharpoons B_3PO_4 + Creatinine \cdot HCl \tag{F}$$
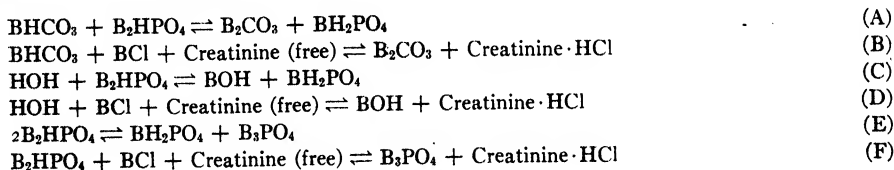
That a definite equilibrium, characterized by the classical constant Keq, exists for each of these reactions can be shown by deriving a general equation that explicitly describes the equilibrium existing among weak acids and bases and their salts in aqueous solution. Employing the convention of Peters and Van Slyke (6), where Ha is a weak acid, bOH a weak base, HA a strong acid, and BOH a strong base:

$$Ha_1 + Ba_{11} \rightleftharpoons Ba_1 + Ha_{11} \tag{I}$$

where
$$Keq = \frac{[Ba_1][Ha_{11}]}{[Ha_1][Ba_{11}]},$$

according to the equation of Henderson (7, 8):

$$[H^+] = Ka_1 \frac{[Ha_1]}{[Ba_1]} = Ka_{11} \frac{[Ha_{11}]}{[Ba_{11}]}.$$

After transposing:

$$\frac{[Ba_1][Ha_{11}]}{[Ha_1][Ba_{11}]} = \frac{Ka_1}{Ka_{11}}$$

$$Keq = \frac{Ka_1}{Ka_{11}} = \text{antilog } (pKa_{11} - pKa_1), \quad \text{or} \quad pKeq = pKa_1 - pKa_{11}.$$

$$b_{11}OH + b_1A \rightleftharpoons b_{11}A + b_1OH \tag{II}$$
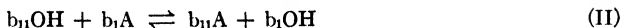
$$Keq = \frac{Kb_{11}}{Kb_1} = \text{antilog } (pKb_1 - pKb_{11}), \quad \text{or} \quad pKeq = pKb_{11} - pKb_1.$$

$$BA + Ha_1 + b_{11}OH \rightleftharpoons Ba_1 + b_{11}A + HOH \tag{III}$$

where BA, like HOH, is sufficiently large to remain rather constant,

$$Keq = \frac{Kab_1}{Kab_{11}} = \text{antilog } (pKab_{11} - pKab_1), \quad \text{or} \quad pKeq = pKab_1 - pKab_{11}.$$

This equation also applies to (I) and (II). p is the negative logarithm of any value. Kab of an acid Ha equals its Ka. Kab of a base bOH equals $\frac{Kw}{Kb}$ where $Kw = [H^+][OH^-] = 10^{-14}$ at 25° C. and $10^{-13.6}$ at 38° C. Kab of an acid or base equals the H^+ ion concentration at half-neutralization of the acid or base. For (I) and (II) as well as (III), where the subscript$_1$ is assigned to the weak electrolyte whose more acid component of its buffer pair is on the left side of the equation, the point of equilibrium is defined by the equation:

$$pKeq = pKab_1 - pKab_{11}. \tag{G}$$

Reactions (A), (C), and (E) are examples of (I), and (B), (D), and (F) are examples of (III). In actual practice, pK values are used. For example, as pK' of $BHCO_3$ is 9.8 and that of BH_2PO_4 is 6.8, tubular reabsorption of B_2CO_3 would drive reaction (A) to the right to keep pKeq = 9.8 — 6.8 (i.e., to maintain Keq at a value of 10^{-3}). The same process would move (B) to the right to keep pKeq of this equation at 9.8 — 4.7 (Keq = 8×10^{-6}). Consequently B_2CO_3 would be constantly reformed while it was being reabsorbed. Similarly reabsorption of BOH would move (C) and (D) to the right; and reabsorption of B_3PO_4 would move (E) and (F) to the right.

If tubular reabsorption can fully account for urinary acidity, the urine, from the standpoint of buffer content and pH, is actually glomerular filtrate minus the reabsorbate. If this is the case, the solution resulting from the addition of reabsorbate to fully elaborated urine should be identical with the glomerular filtrate with respect to its buffer composition and pH. To test the validity of this hypothesis, glomerular filtrates and their corresponding urines were prepared to conform to published analyses (1). To the urines were added reabsorbates calculated to conform to these data. The resulting solutions proved to be identical with the original glomerular filtrates in buffer composition and pH. Presumably this procedure restored to the urine what the kidney had previously removed by reabsorption. Since the calculated reabsorbates contained significant amounts of carbonate, the titratable acidities of the urines under discussion may, therefore, be fully accounted for by

tubular reabsorption of carbonate together with bicarbonate without the necessity of postulating either tubular secretion of acid or exchange in the tubules of H^+ for B^+ ions.   Since the lumen of the renal tubule is extracellular, any buffer equilibria or any relationship between urine, reabsorbate, and glomerular filtrate that can be demonstrated *in vitro* can be expected to exist in the colloid-free, aqueous filtrate in the tubular lumen.

If B_2HPO_4 and BH_2PO_4 are reabsorbed in the ratio in which they occur in the original glomerular filtrate (isohydric reabsorption), such reabsorption does not contribute to titratable urinary acidity.   The less phosphate there is in the remaining tubular urine, the poorer is its buffering power, and hence the more markedly is its pH lowered by $BHCO_3$ or B_2CO_3 reabsorption.

METHODS

The experiment was performed in U-tubes with sufficient mercury in the bottom to keep a solution in one arm completely separate from a solution in the other arm when the tube was in an upright (U) position.   By this means the alkaline reabsorbate containing carbonate and bicarbonate could be prepared in one arm of the tube, while the acid urine was prepared in the other.   The total amount of water used was equal to that contained in urine plus reabsorbate, approximately equal volumes being introduced into each arm.   Equal amounts of phenol red indicator were added to the prepared solutions and the colorimetric standards.   When necessary, additional mercury was added to bring the total volume near the full capacity of the U-tube.   After all visible air-bubbles had been removed, a small supplement of oil (about 1.5 cc. in each arm) was added to permit the insertion of rubber stoppers without the loss of any of the aqueous solution.   No visible air was allowed to remain in the U-tube.   With the tube properly stoppered, it was carefully inverted and the contents judiciously mixed (avoiding emulsification of the oil) until there resulted a uniform color, which was compared with known colorimetric standards (Sörensen phosphate buffers with phenol red indicator in U-tubes of the same dimensions) 0.05 pH apart from each other.

The glomerular filtrate was likewise prepared in a U-tube.   The carbonate and bicarbonate were kept in one arm, separated by mercury from the other arm containing more acidic material.   After the tube was properly sealed and mixed (with phenol red indicator included), the pH of the resulting 'glomerular filtrate' solution was compared with the known colorimetric standards and with the 'urine plus reabsorbate' solution.

Since bicarbonate solutions rapidly lose CO_2 to the air, sodium carbonate was used with an equimolar amount of HCl.   However, in the phosphate experiment, bicarbonate was also used, as an alternate procedure, in preparing the glomerular filtrate and, in conjunction with carbonate, in preparing the reabsorbate.   (See under B of table 1.)   In preparing the glomerular filtrate, carbonate was introduced into one arm, while the HCl was placed in the other.   In preparing the reabsorbate, however, it was possible to put both in the same arm without significant loss of CO_2 if the HCl and buffer substances were added first, followed by the rapid addition of Na_2CO_3 along the wall of the tube.   The thick layer of Na_2CO_3, over the acid-carbonate inter-

face where H_2CO_3 is formed, reacts with any rising CO_2 or H_2CO_3 to form $NaHCO_3$. (But acid layered over Na_2CO_3 will not prevent the escape of CO_2.) The pH of the prepared 'urines' was determined electrometrically. Accuracy in the amounts of B_2CO_3 and HCl used is exceedingly important, since these compounds affect pH most markedly, especially in the creatinine experiment. Sufficient sodium chloride

TABLE 1. PHOSPHATE EXPERIMENT (FIGURES ARE IN mM/MIN.)

Buffer and salt content, and materials used in preparation, of urine, reabsorbate, and glomerular filtrate

	TOTAL CREATININE	CREATININE (FREE)	CREATININE·HCl	BCl	TOTAL Cl	H_2CO_3	$BHCO_3$	B_2CO_3	TOTAL CO_2	$BHPO_4$	B_2HPO_4	TOTAL PO_4	TOTAL B	HCl
A														
1. Filtrate														
a) Calculated	0.171	0.171	0.000	7.516	7.516	0.055	1.304	0.008	1.367	0.102	0.510	0.612	9.958	
b) Ingredients		0.171		6.000					1.367		0.612			1.516
2. Urine														
a) Calculated	0.171	0.159	0.012	0.400	0.412	(0.0)	(0.0)	(0.0)	(0.0)	0.400	0.089	0.489	0.978	
b) Ingredients		0.171		0.000					0.000		0.489			0.412
3. Reabsorbate														
a) Calculated	0.000	0.000	0.000	7.104	7.104	0.000	1.104	0.263	1.367	0.000	0.123	0.123	8.980	
b) Calculated	0.000	0.000	0.000	7.104	7.104	0.000	1.084	0.283	1.367	0.020	0.103	0.123	8.980	
c) Ingredients		0.000		6.000					1.367		0.123			1.104
4. U + R: 'Totals'	0.171				7.516				1.367			0.612	9.958	
5. U + R: Ingredients		0.171		6.000					1.367		0.612			1.516
B														
1. Filtrate														
a) Ingredients	0.171	0.171		7.367	7.516		1.367	0.000	1.367		0.612	0.612	9.958	0.140
2. Reabsorbate														
a) Ingredients	0.000	0.000		7.104	7.104		1.104	0.263	1.367		0.123	0.123	8.890	0.000
3. Urine														
a) Ingredients	0.171	0.171		0.000	0.412		0.000	0.000	(0.0)		0.489	0.489	0.978	0.412
C														
1. Reabsorbate: 0.283 BOH	0.000			7.104	7.104		1.367		1.367	0.020	0.103	0.123	8.980	
2. Reabsorbate: 0.123 B_2PO_4	0.000			7.104	7.104		1.227	0.140	1.367		0.123	0.123	8.980	

NOTES: 'Ingredients' are the source materials used. 'Calculated' content refers to probable distribution of compounds at the pH of the solution. 'U + R' is urine plus reabsorbate. 'Totals' means the totals of the various moieties. In *A*, reabsorbate *a*) has B_2HPO_4 only, while *b*) assumes isohydric PO_4 reabsorption. In *B*, $BHCO_3$ is one of the ingredients used in glomerular filtrate and in reabsorbate. In *C*, *1* is a reabsorbate containing 0.283 BOH, and *2* is one containing 0.123 B_2PO_4. 'Filtrate' is 73.1 cc/min. of glomerular filtrate with pH 7.34 at 38°C. or 7.55 at 22°C. 7.6 cc/min. of urine with pH 6.06 at 22°C. has titratable acidity to pH 7.35 of 0.312 mEq/min. 65.5 cc/min. of reabsorbate has calculated pH of 9.2. Source of essential data is first line of Table 1 of Pitts and Alexander (1).

was added to approximate the chloride concentration of the glomerular filtrate usually encountered in mammals.

The pK'_{ab} of creatinine, little affected by ionic strength, shifts markedly as temperature changes (pK'_{ab} is 5.0 at 22° and 4.7 at 38°C.). BH_2PO_4 has a pK' which is markedly affected by ionic strength or concentration (pK' is 6.8 at ionic strength 0.16) but declines only 0.03 pH as temperature rises from 20° to 38°C.

Data for plasma pH, as reported by Pitts (1), had been obtained by subtracting 0.014 pH per degree difference between room temperature and body temperature (38°C.); the actual pH measurement had been made at room temperature. Since the present solutions were prepared at 22 ± 2°C., they were made to have a pH 0.2 higher than Pitts' published values (1) which were for 38°C.

The titratable acidity of the prepared 'urine' was determined by titrating it to pH 7.35 (determined electrometrically), the end-point used in previous work.

Calculation of reabsorbates was carried out as follows: In the phosphate experiment (see table 1), the glomerular filtrate of pH 7.55 at room temperature (7.34 at 38°C.) had a total PO_4 of 0.612; total creatinine 0.171; total CO_2 1.367; an assumed total chloride of 7.516, obtained by including NaCl to approximate a normal chloride content; and total B of 9.958, part of it due to the NaCl included.

Urine of pH 6.06 at room temperature had total creatinine of 0.171; total phosphate 0.489; negligible total CO_2, Pitts having made no attempt to retain or measure CO_2; an assumed total Cl of 0.410, obtained by including a source of BCl, which helped bring total B to 0.978. Figures are in terms of mM/min.

The reabsorbate, being glomerular filtrate minus urine, therefore contains:

$$\text{Total B} = 9.958 - 0.978 = 8.980$$
$$\text{Total P} = 0.612 - 0.489 = 0.123$$
$$\text{Total } CO_2 = 1.367 - 0.0 = 1.367$$
$$\text{Total creatinine} = 0.171 - 0.171 = 0.000$$
$$\text{Total Cl} = 7.516 - 0.412 = 7.104.$$

Hence, in the reabsorbate, of total B (8.980), 7.104 is BCl, as there is 7.104 Cl. The remaining B, 8.980 − 7.104 = 1.876, may be divided among B_2HPO_4, $BHCO_3$, and B_2CO_3. If the total P is assumed to be in the form of B_2HPO_4, there will be 0.123 B_2HPO_4, accounting for 0.246 B. The remaining B, 1.876 − 0.246 = 1.630, is divided between $BHCO_3$ and B_2CO_3. As total CO_2 is 1.367, there is 1.630 − 1.367 = 0.263 more B than CO_2. Thus there are 0.263 B_2CO_3 and 1.367 − 0.263 = 1.104 $BHCO_3$ in the reabsorbate. This and alternate reabsorbates, including two where isohydric phosphate reabsorption is assumed, are listed in table 1. Similarly calculated reabsorbates for the creatinine are listed in table 2. Any NaCl added does not affect carbonate and bicarbonate content of the reabsorbate.

RESULTS

The data presented in table 1 describe the pH and buffer content of the glomerular filtrate and urine. This is patterned after the pH and buffer content reported on the first line of table 1 by Pitts and Alexander (1) and based on the amounts of such substances needed to prepare such solutions. The data presented in table 2 do likewise with the glomerular filtrate and urine described on the first line of table 2 of the same paper.

The present tables (1 and 2) include the calculated reabsorbates and the materials that can be used in preparing the urines, reabsorbates, and glomerular filtrates. It may be noted that the total CO_2, total phosphate, etc., of the 'urine plus reabsorbate' equal the total for each of these moieties in the 'glomerular filtrate'. Furthermore, these tables show than when Na_2CO_3, HCl, Na_2HPO_4, creatinine, and NaCl are used as source materials, the amount of each of these used for the urine plus the amount of each used for the reabsorbate equals the amount of each used for the glomerular filtrate.

Colorimetric pH determinations showed that the 'urine plus reabsorbate' had the same pH as the corresponding 'glomerular filtrate'. This pH was 7.55 ± 0.03 at room temperature, to correspond to pH 7.34 at 38°C., for the phosphate experiment

(table 1) and 7.45 ± 0.03 at room temperature, corresponding to pH 7.25 at 38°C., for the creatinine experiment (table 2). Room temperature was 22 ± 2°C. An error due to the inclusion of 10 per cent more Na_2CO_3 at the expense of $NaHCO_3$ (keeping total CO_2 constant) would raise the pH of the 'urine plus reabsorbate' 0.09 in the phosphate experiment and about 0.3 in the creatinine experiment. This indicates that the experimental data here presented are correct to within $\pm$ 3 or 4 per cent for the phosphate experiment and within $\pm$ 2 per cent for the creatinine experiment.

TABLE 2. CREATININE EXPERIMENT (FIGURES ARE IN mM/MIN.)

Buffer and salt content, and materials used in preparation, of urine, reabsorbate, and glomerular filtrate

	TOTAL CREATININE	CREATININE (FREE)	CREATININE·HCl	BCl	TOTAL Cl	H_2CO_3	$BHCO_3$	B_2CO_3	TOTAL CO_2	BH_2PO_4	B_2HPO_4	TOTAL PO_4	TOTAL B	HCl
A														
1. Filtrate:														
a) Calculated	1.250	1.247	0.003	9.425	9.428	0.022	0.518	0.002	0.542	0.003	0.015	0.018	9.980	
b) Ingredients		1.250		8.860				0.542			0.018			0.568
2. Urine:														
a) Calculated	1.250	1.103	0.147	2.601	2.748	(0.0)	(0.0)	(0.0)	(0.0)	0.001	0.000	0.001	2.602	
b) Ingredients		1.250		2.600				0.000			0.001			0.148
3. Reabsorbate:														
a) Calculated	0.000	0.000	0.000	6.680	6.680	0.000	0.420	0.122	0.542	0.000	0.017	0.017	7.378	
b) Calculated	0.000	0.000	0.000	6.680	6.680	0.000	0.417	0.125	0.542	0.003	0.014	0.017	7.378	
c) Ingredients		0.000		6.260				0.542			0.017			0.420
4. U + R: 'Totals'	1.250				9.428				0.542			0.018	9.980	
5. U + R: Ingredients		1.250		8.860				0.542			0.018			0.568
B														
1. Reabsorbate: 0.125 BOH	0.000			6.680	6.680			0.542	0.542	0.003	0.014	0.017	7.378	
2. Reabsorbate: 0.017 B_2PO_4	0.000			6.680	6.680		0.437	0.105	0.542			0.017	7.378	

NOTES: 'Ingredients' are the source materials used. 'Calculated' content refers to the probable distribution of compounds at the pH of the solution. 'U + R' is urine plus reabsorbate. 'Totals' means the totals of the various moieties. In A, reabsorbate a) has B_2HPO_4 only while b) assumes isohydric PO_4 reabsorption. 'Filtrate' is 78.7 cc/min. of glomerular filtrate with pH 7.25 at 38°C. or 7.45 at 22°C. 16.5 cc/min. of urine with pH 5.84 at 22°C. has titratable acidity to pH 7.35 of 0.144 mEq/min. 62.2 cc/min. of reabsorbate has calculated pH of 9.25. In B, 1) is a reabsorbate containing 0.125 BOH, and 2 is one containing 0.017 B_2PO_4. Source of essential data is first line of Table 2 of Pitts and Alexander (1).

The titratable acidity of the urine in table 1 (phosphate experiment) is 0.312 mEq/min. to pH 7.35, while that of the urine in table 2 (creatinine experiment) is 0.144 mEq. to pH 7.35. $BHCO_3$ plus B_2HPO_4 reabsorption could, according to Pitts, account for only 33 per cent of the titratable acidity of the urine in table 1 and for only 25 per cent in table 2. Therefore, although there is 3 or 4 times as much $BHCO_3$ as B_2CO_3 in the calculated reabsorbate, reabsorption of B_2CO_3 will account for 2 or 3 times as much of the titratable urinary acidity as will reabsorption of $BHCO_3 + B_2HPO_4$, i.e., 67 per cent in one case and 75 per cent in the other. To account for any titratable acidity reported (1, 3), less than 25 per cent of the total CO_2 reabsorbed need be B_2CO_3, over 75 per cent being $BHCO_3$.

DISCUSSION

Since the pH and the totals of the moieties in the 'urine plus reabsorbate' in tables 1 and 2 are identical with those in the 'glomerular filtrate', the experimental

procedure may be regarded as reversing the reabsorptive processes of the kidney. The results demonstrate that the published data on titratable acidity (1, 3), assuming

TABLE 3. ARTIFICIAL STEP-BY-STEP DESCRIPTION OF CONVERSION OF BUFFER (AND CHLORIDE) CONTENT OF GLOMERULAR FILTRATE TO THAT OF URINE IN PHOSPHATE EXPERIMENT (FIGURES ARE IN mm/MIN.)

Reaction: 1. 2. 3. 4.	$BCl+$ / BCl	H_2CO_3+ H_2CO_3 / $+$	Creatinine $+$ / Creatinine	$=B_2HPO_4$ B_2HPO_4 / $+$	$BHCO_3$ $=BHCO_3$ $+BHCO_3$ / $BHCO_3$	$+BH_2PO_4$ $=BH_2PO_4$ / $-$	Creat·HCl / Creat·HCl $+$	B_2CO_3 / $+B_2CO_3$
Steps:								
'G. F.' A	7.516	0.055	0.171	0.510	1.304	0.102	0.000 / 4×10^{-4}	0.008
B	R7.104			Ro.103		Ro.020		
	0.412	0.055	0.171	0.407	1.304	0.082	0.000	0.008
C		.		−0.275	−0.275	+0.275		+0.275
	0.412	0.055	0.171	0.132	1.029	0.357	0.000	0.283
D	−0.001		−0.001		−0.001		+0.001	+0.001
	0.411	0.055	0.170	0.132	1.028	0.357	0.001	0.284
E								Ro.283
	0.411	0.055	0.171	0.132	1.028	0.357	0.001	0.001
F		+0.118		+0.118	−0.118	−0.118		
	0.411	0.173	0.170	0.250	0.910	0.239	0.001	0.001 / 1×10^{-3}
G					Ro.910			
	0.411	0.173	0.170	0.250	0.000	0.239	0.001	0.001
H	−0.001	−0.011	−0.011		+0.011		+0.011	
	0.400	0.162	0.159	0.250	0.011	0.239	0.012	0.001
J		−0.162		−0.162	+0.162	+0.162		
	0.400	0.000	0.159	0.088	0.173	0.401	0.012	0.001
K					Ro.173			
	0.400	0.000	0.159	0.088	0.000	0.401	0.012	0.001
L				+0.001	+0.001	−0.001		−0.001
	0.400	0.000	0.159	0.089	0.001	0.400	0.012	0.000
M					Ro.001			
Urine	0.400	0.000	0.159	0.089	0.000	0.400	0.012	0.000
Total reabsorbed	7.104			0.103	1.084	0.020		0.283

STEPS: *A.* Composition at room temperature of original glomerular filtrate of pH 7.55 (pH 7.34 at 38°C.). *B.* Reabsorption of 7.104 mM NaCl and isohydric reabsorption of 0.123 mM phosphate. *C, D* and *E.* Reactions 3 and 4 are moved to the right due to reabsorption of 0.283 mM B_2CO_3 even as it is reformed. *F.* Reaction 2 is moved to the left by the excess BH_2PO_4 and $BHCO_3$, reaching equilibrium at pH 6.8 at body temperature. *G, H, J* and *K.* Reactions 1 and 2 are moved to the right due to $BHCO_3$ being reabsorbed even as it is reformed. *L* and *M.* The excess B_2CO_3 moves 3 to the left, reaching equilibrium at pH 6.06 at room temperature after all $BHCO_3$ is reabsorbed. This is 7.60 cc. of urine. (R = reabsorb.) Glomerular filtrate is 73.1 cc.

isohydric phosphate reabsorption, can be fully explained by tubular reabsorption of B_2CO_3 along with $BHCO_3$ without the necessity of tubular secretion of acid or ionic exchange.

Tables 3 and 4 show in artificial step-by-step fashion how the buffer (and salt)

content of the glomerular filtrate of the phosphate and creatinine experiments (respectively) is converted to that of the urine. These tables show that if reabsorption of B_2CO_3 preceded $BHCO_3$ reabsorption but follow isohydric reabsorption of phosphate, the concentration of B_2CO_3 in the remaining tubular urine would, at

TABLE 4. ARTIFICIAL STEP-BY-STEP DESCRIPTION OF CONVERSION OF BUFFER (AND CHLORIDE) CONTENT OF GLOMERULAR FILTRATE TO THAT OF URINE IN CREATININE EXPERIMENT (FIGURES ARE IN mM/MIN.)

Reaction: 1. 2. 3. 4.	BCl+ H_2CO_2 + H_2CO_3 BCl	H_2CO_2 + H_2CO_3 +	Creatinine + Creatinine	= B_2HPO_4 B_2HPO_4 +	$BHCO_3$ = $BHCO_3$ +$BHCO_3$ $BHCO_3$	+ +BH_2PO_4 -BH_2PO_4 =	Creat·HCl + Creat·HCl	B_2CO_3 +B_2CO_3
Steps:								
'G.F.' A	9.425	0.022	1.247	0.015	0.518	0.003	0.003	0.002
B	R6.680			Ro.014		Ro.003		
	2.745	0.022	1.247	0.001	0.518	0.000 2×10^{-4}	0.003	0.002
C	−0.122		−0.122		−0.122		+0.122	+0.122
	2.623	0.022	1.125	0.001	0.396	0.000	0.125	0.124
D				−0.001	−0.001	+0.001		+0.001
	2.623	0.022	1.125	0.000	0.395	0.001	0.125	0.125
E								Ro.125
	2.623	0.022	1.125	0.000	0.395	0.001	0.125	0.000
F	+0.105	+0.105	+0.105		−0.105		−0.105	
	2.728	0.127	1.230	0.000 3×10^{-4}	0.290	0.001 7×10^{-4}	0.020	0.000 1.4×10^{-4}
G					Ro.290			
	2.728	0.127	1.230	0.000	0.000	0.001	0.020	0.000
H	−0.127	−0.127	−0.127		+0.127		+0.127	
	2.601	0.000	1.103	0.000	0.127	0.001	0.147	0.000
J					Ro.127			
Urine:	2.601	0.000	1.103	0.000 1×10^{-4}	0.000	0.001 9×10^{-4}	0.147	0.000
Total reabsorbed	6.680			0.014	0.417	0.003		0.125

STEPS: *A*. Composition at room temperature of original glomerular filtrate of pH 7.45 (pH 7.25 at 38°C.). *B*. Reabsorption of 6.680 mM NaCl and isohydric reabsorption of 0.017 mM phosphate. *C, D* and *E*. Reactions 3 and 4 are moved to the right due to reabsorption of 0.125 mM B_2CO_3 even as it is reformed. *F*. Reaction 1 is moved to the left by the excess $BHCO_3$ and creatinine·HCl; equilibrium is reached at pH 6.5 (at 38°C.). *G, H* and *J*. Reaction 1 and 2 are moved to the right due to $BHCO_3$ being reabsorbed, even as it is reformed, the result being 16.5 cc. of urine at pH 5.84 at room temperature (R = reabsorb.) Glomerular filtrate is 78.7 cc.

the end of B_2CO_3 reabsorption, be 0.7 $\times$ 10^{-4} Molar (assuming 15 cc. of urine remains) at pH 6.8 in the phosphate experiment and 0.7 $\times$ 10^{-5} Molar (assuming 20 cc. of urine remains) at pH 6.5 in the creatinine experiment. If reabsorption of B_2CO_3 and $BHCO_3$ began together and proceeded at the same rate (but followed isohydric phosphate reabsorption), the concentration of B_2CO_3 in the tubular urine at the completion of $B_2'CO_3$ reabsorption would be 0.5 $\times$ 10^{-4} Molar (at pH 6.75 in 12 cc. of urine remaining) in the phosphate experiment and 0.3 $\times$ 10^{-5} Molar (at pH 6.3 in
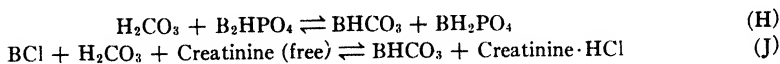
20 cc. of urine remaining) in the creatinine experiment.   Since 10^{-5} Molar is the approximate concentration of $BHCO_3$ in urines of low pH, there should be no more difficulty in accepting the view that reabsorption of B_2CO_3 can occur than in accepting the widely held view that reabsorption of $BHCO_3$ (8, 14) can occur.   Indeed these concentrations (0.7×10^{-5} to 0.7×10^{-4} Molar) of B_2CO_3 are about 200 to 2000 times the concentration in blood of the H^+ ion (about 0.4×10^{-7} Molar), which is involved in the ionic exchange.   (These statements also apply to the urines with the highest titratable acidity and lowest pH reported (1, 3) by Pitts.)   Hence, if probability parallels concentration, reabsorption of B_2CO_3 (with $BHCO_3$) is, on the basis of known concentrations, a more probable explanation of the titratable urinary acidity than is ionic exchange.

Ionic exchange of $1B^+$ for $1H^+$ ion has virtually the same effect on the buffer content and pH of the tubular cell and of the tubular urine as the reabsorption of 1 molecule of BOH.   The resulting formation of considerable H_2CO_3 or CO_2 in the tubular urine has not been mentioned in any of the diagrams or discussions (1, 2, 3, 9) of the acidification of the urine by ionic exchange, which is, therefore, not so simple and direct a process as appears at first sight.   The H_2CO_3 or CO_2 thus formed is presumed (4) to diffuse slowly into the tubular cells.   This is tantamount to the extreme separation of the transferred materials into $BOH + H_2CO_3$ or $2BOH + H_2CO_3$ (instead of $BHCO_3$ or B_2CO_3).   Since the presumed diffusion of H_2CO_3 or CO_2 out of the tubular urine often lags behind ionic exchange, this mechanism would have a more jarring effect on tubular cell pH than reabsorption of an equivalent amount of $BHCO_3$ or B_2CO_3.

Theoretically, reabsorption of 1 molecule each of $BOH + BHCO_3$ could, as shown in tables 1 and 2, have the same effect as the reabsorption of 1 molecule of B_2CO_3. This seems a less likely process, however, for several reasons: *a*) the concentration of B_2CO_3 in the filtrate far exceeds that of BOH; *b*) the heat content and free energy content of $B_2CO_3 + H_2O$ are considerably less than those of $BOH + BHCO_3$; *c*) the minimum osmotic work required for the reabsorption of $B_2CO_3 + H_2O$ at the pH of the tubular contents when this process occurs is less than that required for the reabsorption (transfer) of $BOH + BHCO_3$.   Reabsorption of $B_3PO_4 + BHCO_3$ could not fully account for the urinary acidity in these experiments because of the small amount of phosphate reabsorbed.   Where infusions of phosphate or creatinine have not been employed, reabsorption of $BHCO_3$ (carbonic acid filtration theory) can fully explain urinary acidity (3).

Reabsorption of B_2CO_3 might be compared with the ionic exchange mechanism on the basis of probability.   Within cells, the concept of ionic exchange can be accepted without serious objections.   Similar exchanges, against a concentration gradient, between cellular contents and tubular fluid that is exteriorized present conceptual difficulties.   This, of course, is no insurmountable obstacle.   From the standpoint of chemical kinetics and thermodynamics, however, reabsorption of B_2CO_3 would have an advantage in economy and would seem more probable, because the concentration of this salt in the glomerular filtrate, though small, is enormously greater than that of H^+ ion in the plasma and because the minimum osmotic work required for the reabsorption (transfer) of B_2CO_3 is less than that required for the transfer of H^+ ions in ionic exchange or in the secretion of molecular acid.

The observation (10) that very high concentrations (tensions) of CO_2 can be attained in the urine has been interpreted by some as evidence of the impermeability of the tubules to ready diffusion of CO_2. If diffusion of CO_2 occurred, it would be in both directions across the luminal membrane, the net direction and rate varying with circumstances. Any CO_2 (H_2CO_3) diffusing into the tubular urine would enter into the equilibrium reactions:

$$H_2CO_3 + B_2HPO_4 \rightleftharpoons BHCO_3 + BH_2PO_4 \tag{H}$$
$$BCl + H_2CO_3 + Creatinine\ (free) \rightleftharpoons BHCO_3 + Creatinine \cdot HCl \tag{J}$$

Pitts (1) considers the tubules readily permeable to the diffusion of CO_2. This assumption renders unnecessary his postulation of *active* transport of acid, such as secretion of acid or ionic exchange against a concentration gradient, because if the CO_2 (H_2CO_3) concentration of the tubular urine fell below that of the surrounding peritubular fluids and carbonic anhydrase-rich tubular cells, CO_2 (H_2CO_3) would *diffuse* into the urine. Consequently, reabsorption of $BHCO_3$, as postulated by Peters and Van Slyke (6) and by Sendroy, Seelig, and Van Slyke (11), could fully explain Pitts' experimental results (carbonic acid filtration-diffusion theory). The only active renal function here would be reabsorption of $BHCO_3$. However, if the tubules were not sufficiently permeable to the ready diffusion of CO_2, enough $BHCO_3$ could not be formed therefrom (by buffer equilibria) and reabsorbed within a given period to produce the titratable acidities of Pitts' experiments. Regardless of the degree of tubular permeability to CO_2, the reabsorption of B_2CO_3 could occur.

Indirect evidence, adduced in favor of active transport of acid, also favors active transport (reabsorption) of $BHCO_3$ and B_2CO_3. The effect of sulfanilamide in decreasing urinary acidity (1, 3, 12) may be attributed to its effect on carbonic anhydrase and various enzyme systems involved in $BHCO_3$ and B_2CO_3 reabsorption. Calculations of published data (12) show that $BHCO_3$ reabsorption is decreased by sulfanilamide. The same may be true of B_2CO_3 reabsorption. Furthermore, if B_2CO_3 is reabsorbed, its subsequent reaction with H_2CO_3 could be slowed owing to a decreased rate of H_2CO_3 formation from CO_2 during inhibition of carbonic anhydrase activity by sulfanilamide. An accumulation of reabsorbed B_2CO_3 would cause an abnormal rise in cellular pH, which could impair certain reabsorptive activities. Davenport (13) has withdrawn his hypothesis that carbonic anhydrase is of significance in the secretion of gastric acid. Its function in the kidney is not definitely known.

The hypothesis (1, 3, 14) that the ability of the kidney to excrete acid is determined by its ability to transfer H^+ ions against a concentration gradient, is weakened considerably by the fact that more acid was eliminated both by man (3) and the dog (1) in urines with a pH of 5.54 and 5.61 respectively, representing a H^+ ion concentration of about 70 to 1 (as compared to serum pH), than in urines of much lower pH, e.g. 4.5, representing a gradient of 800 to 1. The urines containing more titratable acid had more buffer than the urines of lower pH. The greater titratable acidity observed (1, 3, 9, 14) when more buffer is present or when a buffer with a higher pK'_{ab} is infused can be readily explained by the fact that, under these circumstances, more $BHCO_3$ and B_2CO_3 can be reabsorbed before their concentrations fall to any given level.

The evidence of Conway *et al* (15) that the proximal tubule is impermeable to sodium does not support the view (1) that four fifths of the filtered $NaHCO_3$ and H_2CO_3 undergo isohydric reabsorption in the proximal tubule. Furthermore, up to 30 per cent of the filtered water appeared in the urine in the experiments on urinary acidification (1).

SUMMARY

Experiments are presented to show that because of buffer equilibria, for which an equation is derived, sufficient B_2CO_3 can be reabsorbed in addition to $BHCO_3$ to produce any titratable urinary acidity yet reported. Physico-chemical and other considerations favoring such a mechanism are discussed. Conditions under which active tubular reabsorption of $BHCO_3$ can fully account for titratable urinary acidity are also noted.

High titratable acidity of the urine can be completely explained as well 1) by active tubular reabsorption of alkaline compounds whether or not the tubular luminal membrane is permeable to the diffusion of CO_2 (H_2CO_3), as 2) by active tubular transport of acid (either ionic exchange or secretion of acid) into the tubular lumen followed by passage of CO_2 (H_2CO_3) out of the tubular lumen, or 3) by some combination of 1) and 2).

The mechanism responsible for the production of urinary acidity has not yet been conclusively demonstrated.

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PLASMA INORGANIC PHOSPHATE CONCENTRATION AND HYPERVENTILATION IN THE DOG[1]

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HYPERVENTILATION in man is known to result in a lowering in plasma inorganic phosphate concentration (1, 2, 3) and in urinary phosphate excretion (3, 4). Anesthesia (5, 6) and shock (7) are associated with plasma concentration changes in the reverse direction. In order to be able to study the fate of phosphate retained in the body and lost from the plasma in hyperventilation it seemed important to study the problem in experimental animals. It is technically difficult to hyperventilate unanesthetized dogs mechanically while their respiratory motor mechanisms are functional. This paper is a report of studies on the effects of hyperventilation of dogs under general anesthesia or without anesthesia, employing curare in the latter case to permit overventilation.

METHODS

Mongrel dogs without special pre-treatment were used. For the experiments under general anesthesia nembutal 30 mgm/kgm. was administered intravenously. Artificial respiration through a tracheal tube was administered by means of a variable speed, variable stroke pump. The arterial blood CO_2 content was measured by the manometric method of Van Slyke and Neill (8). The plasma inorganic phosphate was determined by the method of Fiske and SubbaRow (9). In the experiments with curare 0.6 to 1.0 unit of curare (Intocostrin, Squibb) per pound of body weight brought about respiratory paralysis.

RESULTS

Observations on 11 dogs are presented in table 1. In the first two sections of the table the effects of hyperventilation under nembutal and under curare are contrasted. It is apparent that in nembutalized dogs no appreciable change in plasma inorganic phosphate concentration is produced by hyperventilation for periods up

TABLE 1. INFLUENCE OF HYPERVENTILATION ON PLASMA INORGANIC PHOSPHATE IN RELATION TO ANESTHESIA

DOG NO.	ANESTHETIC	CURARE	DURATION OF HYPERVENTILATION	CO₂ CONTENT	INORGANIC PO₄
			min.	*vol. %*	*mgm. %*
1	Nembuta	—	0	53.8	3.3
	"	—	40	37.9	3.1
2	"	—	0	41.1	4.2
	"	—	60	34.2	4.0
3	"	—	0	40.8	3.5
	"	—	120	26.1	3.4
4	"	—	0	46.2	6.9
	"	—	300	27.6	6.6
5	None	+	0	34.8	4.7
	"	+	75	14.6	0.6
6	"	+	0	54.5	3.0
	"	+	90	38.9	0.7
7	"	+	0	35.1	3.0
	"	+	120	24.7	0.6
8	"	+	0	34.1	4.0
	"	+	120	22.0	0.7
9	Local	+	0	43.2	2.8
	"	+	40	40.6	1.7
	"	+	240[1]	36.0	4.2
	"	+	300	33.6	5.6
10	None	—	0	48.0	4.5
	"	—	(30)[2]	47.4	4.2
11	"	—	0	44.5	4.6
	"	—	(60)[2]	44.4	4.1

[1] Severe hemorrhage in the interval preceeding.
[2] Quiet spontaneous breathing of trained dogs lying supine.

to five hours and of a degree sufficient to reduce the arterial blood CO₂ content by a third. By contrast, without anesthesia the same lowering in arterial CO₂ content by hyperventilation is associated with a decline in plasma inorganic phosphate to a fourth or less of its initial value.

When surgical procedures under local anesthesia are associated with hyperventilation in the curarized dog the changes in plasma inorganic phosphate are not uniform. An example of four such experiments is shown in table 1 in the case of

dog 9. It will be seen that following hemorrhage the plasma inorganic phosphate level rose.

To determine whether simple restraint in the dorsal recumbent posture (10) would produce changes in plasma inorganic phosphate comparable to those seen in hyperventilation, *dogs 10* and *11* were so treated. Minimal alterations in the measured plasma constituents occurred.

DISCUSSION

The marked decline in plasma inorganic phosphate observed in voluntary (1, 2) or passive (3) hyperventilation in man can be observed also in the dog, if the complications of anesthesia, hemorrhage and shock are obviated. It is important that this point be established because it indicates that the phenomenon is not peculiar to one species, and further because a method is now available for study of the mechanism in experimental animals. This point acquires significance because chronic passive hyperventilation in man (3) results in a net storage of phosphate in the body. The site of that storage may be important in connection with the changes in respiratory function, particularly in sensitivity to the alveolar CO_2 tension, observed after chronic hyperventilation (11).

CONCLUSIONS

Plasma inorganic phosphate concentration was markedly decreased by artificial overventilation of unanesthetized dogs which had received intravenous curare to the point of respiratory paralysis. Hyperventilation of anesthetized animals failed to lower plasma inorganic phosphate. Overventilation of dogs in shock resulted in irregular changes in plasma inorganic phosphate. The restraint of quiet, well trained dogs in the supine position produced only a slight fall in plasma inorganic phosphate.

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CHRONIC INANITION, RECOVERY, AND METABOLIC RATE OF YOUNG RATS

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I T HAS been shown that adult organisms on restricted diets adjust themselves by a reduction in basal metabolism. Klemperer (1), Svenson (2), and Magnus-Levy (3) after clinical observations hypothesized that there is such an adjustment to low caloric diets. Castaldi (4), as a war prisoner, spoke of 'adaptation' to reduced diets. The experimental observations of Pashutin (5) on animals maintained with small rations showed a reduction in respiratory oxygen and carbon dioxide. Morgulis (6) concluded that under the condition of chronic inanition the basal metabolism passes through the same stages of depression recognized in acute inanition. Keys (7) found that men underfed for 24 weeks exhibited a decline of 30 per cent in basal metabolism, or a decline of 10 per cent if calculated per unit weight of active tissue.

Investigations on the metabolic rate in recovery from chronic inanition have been limited to adult animals. Morgulis (6), experimenting with a dog, found that after two weeks of realimentation the metabolic rate increased 100 per cent over that of chronic inanition and 30 per cent above normal. The values did not drop to normal until after five weeks of recovery feeding. Keys *et al.* (8) reported that after 12 weeks of rehabilitation of semi-starved men the basal oxygen consumption was still considerably below the control; after 20 weeks it was slightly higher than the control; and after 32 weeks it was nearly the same as the control. The recovery was closely determined by the caloric intake.

No study has been made of respiratory metabolism during chronic inanition and recovery in young growing animals. It was the purpose of this investigation to determine the effects of chronic underfeeding on the metabolic rate and the respiratory quotient and to follow the course of metabolic recovery during realimentation. Also presented in this report are the alterations in metabolism produced by therapeutic injections of growth hormone, testosterone, and B-complex administered during the recovery period.

METHODS

Carbon dioxide is an end product of oxidation of all carbonaceous material, aside from those fragments of the protein molecule that are excreted in the urine. A measure of the carbon dioxide production furnishes, therefore, an estimate of total catabolism. Because of the simplicity and consequent accuracy, the metabolic rates in this experiment were calculated from carbon dioxide determinations.

The studies were arranged in two separate experiments. In both experiments young male albino rats, 30 days of age, were employed in groups of 10, each group constituting an experimental

unit. The rats were placed in individual cages and were underfed by restriction of the daily intake of a qualitatively balanced ration to an amount that would just maintain body weight. At the end of the selected periods of underfeeding the animals were put upon full and adequate rations for various periods of time.

Sample metabolic rates were taken of the rats in a state of chronic inanition of 30 days' duration, and after 10, 20, and 30 days of refeeding. Similar determinations were made on other animals after a more prolonged underfeeding of 90 days and at refeeding intervals of two, five, and eight weeks. Determinations were also made on fully fed normal rats. All metabolic data collected were non-fasting; that is, food was not withheld 18 hours before determinations.

A modification of the Haldane open-circuit was employed in making these metabolic studies. Air was drawn through the animal chamber at a rate of 2 l/min. after being made carbon dioxide and water free by passing it through moist soda lime, anhydrous calcium sulfate and activated alumina. Carbon dioxide was collected from the air leaving the animal chamber for a period of two to four hours by the use of Ascarite. Water in the air was collected by the use of a Swartz tube immersed in a bath of dry ice and alcohol. The collecting tubes were weighed on an analytical balance to the nearest milligram, and the cage, with animal, feces and urine, was weighed on a torsion balance to the nearest hundredth gram. Movement of animals was kept at a minimum by the use of bright lights and by permitting the rats to quiet down in the chamber before making the determination. The experiments were conducted during the winter and the determinations were made between 10:00 A.M. and 4:00 P.M. in accordance with Horst *et al.* (9), who showed that oxygen uptake in the rat is abnormally high in the morning and late afternoon, and in accordance with Sherwood (10), who reported that rats exhibit a marked diminution in metabolism during the summer months. The metabolic rates are expressed as the number of calories liberated per square meter of body surface in 24 hours, the latter being calculated as the two-thirds power of the body weight multiplied by 9.1 as a constant. The calories were calculated from an average respiratory quotient based upon preliminary experiments and from the amount of carbon dioxide released.

The administration of growth hormone and testosterone has been described in a previous report by Quimby (11). Vitamin B complex was given daily by intraperitoneal injection in the amount of 0.2 ml. The preparation employed was 'Betalin Complex' (Lilly) and contained in each injection 0.5 mg. thiamin, 0.2 mg. riboflavin, 0.25 mg. pantothenic acid and 0.5 mg. pyridoxine.

RESULTS

TABLE 1. EFFECT OF VARIOUS PERIODS OF REFEEDING AND RECOVERY THERAPY ON THE METABOLIC RATE OF THE RAT[1] (CALORIES PER 24 HOURS PER SQUARE METER BODY SURFACE). MEAN AND STANDARD DEVIATION.

EXPER.[2] GROUP	REFED 10 DAYS	REFED 20 DAYS	REFED 30 DAYS
Saline control	1710 ±42	1375 ±36	1330 ±54
Growth hormone	1395 ±68	1260 ±81	1150 ±49

EXPER.[3] GROUP	REFED 2 WEEKS	REFED 5 WEEKS	REFED 8 WEEKS
Saline control	1752 ±32	1517 ±50	1322 ±57
Growth hormone	1382 ±12	1414 ±133	1274 ±136
B-complex	1710 ±58	1496 ±81	1334 ±70
Testosterone	1402 ±78	1466 ±24	1354 ±16

[1] Each group in the tables consisted of 10 animals.

[2] Before refeeding, these rats had an average metabolic rate of 1014 ±96 as a result of a 30-day chronic starvation period.

[3] Before refeeding, these rats had an average metabolic rate of 920 ±102 as a result of a 90-day chronic starvation period. The average metabolic rate of fully-fed normal rats was 1149 ±125.

DISCUSSION

Effect of Chronic Inanition on Metabolic Rate.   Table 1 shows that there was about a 10 per cent and 20 per cent reduction in the metabolic rate after 30 days and 90 days of semi-starvation respectively.   The caloric restriction employed in this experiment in bringing young rats to a state of chronic inanition was accompanied by a proportional restriction of minerals and vitamins.   The amount of thiamin (vitamin B_1) contained in the daily ration during the underfeeding period was 0.02 milligram.   This constituted, according to Griffith and Farris (12), only about one fourth of the minimum daily requirements necessary for normal growth.   Although the cause of the depressed metabolism in the underfed rats was not investigated in this experiment, the studies of other workers (13–20) suggest that it may be attributed to the specific deficiency of vitamin B_1, the effect being mediated through the pituitary and thyroid glands.

Effect of Chronic Undernutrition on the Respiratory Quotient.   The respiratory quotients of these semi-starved rats were, without exception, low, typically fasting values in determinations made before permitting the daily ration; and they were high, typically non-fasting values in determinations made after consumption of the daily ration.   The mean of six fasting determinations was 0.75 and that of six non-fasting determinations was 0.94.   Although the type of food restriction in this experiment was purely quantitative, with carbohydrates, fats and proteins available in balanced but inadequate amounts, one might have expected a reduction in the R. Q. on the basis of other studies.   For example, Dann and Chambers (21) found an almost complete suppression of the ability to oxidize ingested glucose in dogs after a three-week fast.   Chambers (22) noted that as the amount of carbohydrate in the diet was decreased there was a diminished utilization.   Marrazzi (23) observed that restriction of food intake decreased absorption of glucose.   It is clear, however, that in the young semi-starved rats of this present experiment carbohydrates were being normally if not preferentially absorbed and metabolized as shown by the high respiratory quotients found in determinations made following the ingestion of food.   In fact, Quimby (24) has shown that absorption and utilization of food in these animals was actually facilitated by chronic starvation.

Effect of Refeeding on Metabolic Rate.   The stimulation of metabolism which occurred in early recovery (table 1) must be associated with the intense growth and regeneration of the active protoplasm.   The influx of nitrogenous materials which naturally accompanied realimentation might offer an explanation, but this does not appear reasonable in view of the fact that the supply of nourishment continued to be great even as the metabolic rate gradually returned to normal.   It is clear therefore that the enhanced metabolism of recovery must be interpreted as due to the active, growing, regenerating tissues.   There is no evidence in the literature and no measurements were taken in this experiment which would indicate that the pituitary or thyroid were responsible for this phenomenon.

The metabolic rate was nearly normal at the end of the refeeding periods, although the longer period of undernutrition prolonged the time required for recovery.

Effect of Growth Hormone and Testosterone on the Metabolic Rate During Refeeding.   The reduction in the metabolic rate during recovery which resulted from growth

hormone injections (table 1) is in agreement with the results obtained on normal animals with similar growth extracts used by Kleiber and Cole (25) and Teel and Cushing (26), whose extracts reduced metabolism while promoting growth. The explanation of this effect, however, must await the evidence of further experimentation.

Injections of testosterone also resulted in a reduction of metabolic rate (table 1). The action in this case was due to the inhibitory effect of the sex hormone on the pituitary gland. In a previous report, Quimby (11) has shown that pituitary depression resulted from the testosterone therapy employed in these animals.

The fact that the testosterone and growth hormone did not maintain their depressing action on the metabolic rate throughout recovery was probably due to the fact that the pituitary recovered in its secretory capacity so as to counteract the inhibitory effects of these substances.

The administration of vitamin B complex was without effect, indicating that amounts adequate for metabolic recovery were present in the food consumed during realimentation.

SUMMARY

Chronic starvation in young growing rats lowered the metabolic rate 10 to 20 per cent below normal, but an abnormally high metabolism appeared during the early period of refeeding. The enhanced metabolism of early recovery was decreased in rats treated with growth hormone and with testosterone.

The administration of vitamin B complex did not alter the course of metabolism during recovery. The metabolic rates of all groups were normal or nearly normal at the end of the refeeding periods, although the longer period of undernutrition prolonged the time required for recovery. The non-fasting respiratory quotients of the rats in a state of chronic undernutrition had values slightly higher than those given for normal rats.

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EFFECTS OF ALTITUDE ANOXIA ON RENAL FUNCTION

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AT THE present time there is a paucity of data available on the effect of reduced atmospheric pressure on the excretory function of the kidney. Alving et al. (1) studied the effects on renal function of exposure of young men to simulated altitudes of 10,000 to 18,000 feet for four to six hours daily, six days per week over periods of four to six weeks. They found that such exposure caused no change in renal plasma flow, glomerular filtration rate nor filtration fraction, but did cause invariably an increase in maximum tubular excretory capacity for diodrast (Tm_D). In none of eight experiments performed on 5 subjects was a decreased Tm_D observed. In 2 subjects at 10,000 feet and 11,500 feet, respectively, the increase was within the standard deviation for normal subjects at ground level, but in all other experiments the increases were of greater magnitude. An increased Tm_D was noted on the thirteenth day of exposure to altitude, the earliest that any subject was studied. In 2 subjects the Tm_D showed no tendency to return to pre-ascent values for as long as 41 days, the longest time that studies were made at altitude.

The oxygen requirement of the normal kidney has been studied by several investigators. In 1905, Barcroft and Brodie (2, 3) reported the oxygen consumption per minute by single-kidney dogs varied widely but averaged about 1.5 cc., and that diuresis produced by urea and sodium sulfate was accompanied by a large increase of the oxygen absorbed by the kidney. They did not believe that the increased oxygen consumption was attributable to the increased urine output at the glomerulus but stated that, ". . . it is under these circumstances that the kidney may be most active". Fee and Hemingway (4), using perfused isolated kidney preparations, also reported a rise in renal oxygen consumption with increased urine volume. Hayman and Schmidt (5) reported results of oxygen consumption by the kidney of anesthetized dogs on the cubic centimeters of oxygen per gm. per minute basis similar to those of Barcroft and Brodie, but did not notice any characteristic effect on the apparent oxygen metabolism of the kidney by increased urine volumes produced with caffeine or sodium sulfate. Adolph (6) reported that in frog kidneys a 4 per cent atmosphere of oxygen was sufficient to cause a complete cessation of urine formation. This effect was shown to be due to constriction of the renal arterioles and was not modified by denervation of the kidney. Van Slyke et al. (7) were unable to show any relationship of renal blood flow or oxygen consumption to the work of the kidney in excreting urea or water at greatly varying rates. The renal oxygen consumption of the dogs used in their studies varied from 2.0 to 13.3 cc. of O_2 per minute for two-kidney dogs, and 1.1 to 9.5 cc. in one-kidney animals. Thus it is seen that in all *in vivo* studies of renal oxygen consumption reported considerable variations are noted. In regard to the problem of oxygen requirement for excretory work of the kidney Van Slyke et al. stated: "It appears that neither the excretory work nor the processes directly connected with it control the oxygen consumption of the kidney, which must be governed by the energy requirements of the non-excretory processes in the organ. This conclusion is explicable by the fact that, as calculated by Brodie, Barcroft and others, the thermodynamic work ordinarily done by the kidney in excretion is less than one per cent of the energy furnished

by the respiration of the organ." These same investigators observed that, in view of the fact that the renal venous blood is normally more than 85 per cent oxygenated, the oxygen tension under which the tissues are maintained is higher than in most other organs.

Toth (8) noted that anoxia in anesthetized dogs induced by the respiration of gas mixtures containing percentages of oxygen below 10 per cent for $\frac{1}{4}$ to $2\frac{1}{2}$ hours usually resulted in oliguria, but occasionally in polyuria. By giving infusions of epinephrine to dogs he could produce either oliguria or polyuria depending upon the rate of the infusion. In view of the similar effects of anoxia and epinephrine on the urinary output, Toth proposed that epinephrine was the responsible agent in anoxia for the observed oliguria and polyuria.

Malmejac (9) reported on the effect of anoxia on kidneys explanted to the neck and interposed on the caroticojugular circulation in dogs. He noted that urine secretion began to decrease noticeably at reduced atmospheric pressures equivalent to altitudes between 14,000 and 18,000 feet. At 24,000 feet urine secretion was three quarters of the usual amount. At from 30,000 to 33,000 feet renal blood flow and urine secretion ceased entirely and terminal cardiovascular accidents appeared.

Recent studies (10–14) demonstrate a remarkable resistance of the kidney to prolonged ischemic anoxia with a striking ability of the kidney exposed to recover its normal functions within a short time. Studies based on reduction of renal blood flow are strongly indicative of marked inhibition of the oxidative systems in the kidney under these conditions.

Using the Warburg technique for measuring cellular respiration, it has been demonstrated that the QO2 (cubic millimeters of oxygen per mg. of dry weight of tissue per hour) of kidney tissue is exceeded only by that of the retina (15–17). This is good indirect evidence that the kidney has a very high oxygen requirement. In studies using the Warburg technique in our laboratories the effect of reduced atmospheric oxygen on the QO2 of kidney slices was determined (18). In these studies kidney tissue slices 0.3 mm. thick were subjected to atmospheric oxygen concentrations of six per cent in the Warburg apparatus. The QO2 measured in this situation was two thirds of the QO2 in air. In this respect the kidney shows no difference from liver and heart muscle which have similar reductions of QO2 under the same reduced oxygen tensions. These findings are *educed* to show that although the kidney may have a remarkable ability to maintain its viability under conditions of anoxia, the overall work ability of this organ is not necessarily maintained. Indeed, from the *in vitro* evidence it is conceivable that a lowering of maximum work ability is effected.

The above described studies on the relationship of oxygen requirements of the kidney to the function of this organ may be epitomized as follows. *a*) Oxygen consumption of the kidney varies widely within the course of an experiment and does not seem to bear a characteristic relationship to excretory work of this organ. *b*) The oxygen consumption of the kidney is determined primarily, and almost exclusively, by non-excretory processes of the kidney. *c*) The high degree of oxygen saturation of renal venous blood (85% or more) indicates that renal tissues are normally maintained under higher oxygen tension than most other organs. *d*) Under conditions of anoxia the urinary output is usually decreased but may be occasionally increased. However, as one approaches a critical level of atmospheric oxygen tension (about 45 mm. Hg in dogs) renal blood flow and urine formation cease and the animal is in a moribund state. *e*) The normal kidney is remarkably resistant to rather prolonged periods of anoxia. This ability of the kidney to maintain its viability under prolonged periods of anoxia is coupled with the marked inhibition of oxidative systems in the kidney. However, it should be stated that the overall work ability of the kidney under this anoxic condition is not known.

EXPERIMENTAL PROCEDURE

Five well trained dogs, divided into two groups, were employed in these studies. All animals were unanesthetized and loosely restrained on a comfortable animal board.

The dogs were fasted for 12 hours before the experiment and were given 50 cc. of water per kg. of body weight one or two hours before the collection periods were begun. An indwelling, mushroom catheter was used in obtaining all urine specimens. Near the end of each collection period the bladder was washed once or twice with 20 or 40 cc. of saline and a comparable volume of air. Manual expression of the bladder was used in conjunction with the saline-air flushings. An additional aid in insuring complete bladder evacuation was the positioning of the animal board on an incline of about 20° with the head elevated. The urine collection periods ranged from 10 to 15 minutes each. Blood was obtained by external jugular puncture and heparin was used as an anticoagulant. The renal function tests were performed at ground level and in an altitude chamber at simulated altitudes of 18,000 feet (79.4 mm. Hg oxygen tension) and 24,000 feet (61.6 mm. Hg oxygen tension).

In *experiment I*, 3 adult, female, mongrel dogs, each weighing about 10 kg., were used. In this experiment creatinine was used to measure the glomerular filtration rate (GF) and para-aminohippuric acid (PAH) was used to measure the effective renal plasma flow (C_{PAH}) and the maximum tubular excretory ability (Tm_{PAH}). At least 20 minutes before the first period a primer dose of creatinine and PAH was given intravenously and immediately after this an intravenous infusion of creatinine and PAH was begun.

In the creatinine determination a 'correction' based on the initial plasma blank was not used, as the preponderance of data in the literature suggests that endogenous creatinine (creatinine plus other substances giving the Jaffe reaction) is treated quite similarly to exogenous creatinine by the kidney. Any error that might arise from omission of the 'correction' was minimized by maintaining the plasma creatinine levels above 10 mg. per cent in all cases as advocated by Shannon *et al.* (19). Plasma PAH values were maintained between 1 and 3 mg. per cent for measurement of C_{PAH} and between 30 and 90 mg. per cent for determination of Tm_{PAH}.

The following procedure was adhered to for the values obtained at ground level (750 feet above sea level) and at simulated altitudes of 18,000 feet and 24,000 feet. Eight urine collections were carried out on each dog, starting five minutes after reaching the simulated altitude. During the first several periods creatinine clearance and PAH clearances were obtained. Each animal was then given a booster dose of PAH intravenously and a new infusion of higher PAH concentration was substituted for the original infusion solution. After 20 to 30 minutes several more urine collection periods were carried out to determine the Tm_{PAH} values. Thus the elapsed interval of time between the time of reaching the desired altitude and the time of commencing the first of the series of consecutive urine collection periods for determination of Tm_{PAH} was in all cases approximately 75 minutes. A minimum interval of one week was maintained between successive tests on any one animal, and each dog was tested at only one altitude at any one time.

Experiment II was designed to test the changes attributable to altitude by studies in which measurements were made at different altitudes on the same day.

In this group the measurement of the effective renal plasma flow was omitted in order that the experimental procedure might adhere to the following pattern. The Tm_{PAH} was determined for several consecutive urine collection periods at ground

level pressure in the altitude chamber. Immediately after the last period, the pressure was reduced to a simulated altitude of 18,000 feet. The rate of ascent was standardized at 3000 feet per minute. Five minutes after reaching this altitude the animals were subjected to several more urine collection periods. Immediately after the last period the pressure in the chamber was further reduced to simulate an altitude of 24,000 feet. Here also ascent was at the rate of 3000 feet a minute. Again, several consecutive urine collection periods were carried out five minutes after reaching this altitude.

Two adult, female, Dalmatian coach hounds, each weighing about 22 kg. comprised *group 2*. In this group inulin was used rather than creatinine to measure the glomerular filtration rate, as we have reason to doubt that creatinine is a completely reliable expression of glomerular filtration in Dalmatian coach hounds (20). The inulin was prepared for intravenous administration in the manner prescribed by Smith *et al.* (21). To insure adequate urine flow for the purpose of avoiding crystallization of the inulin in the urine, the infusion was made to contain sodium sulfate in a concentration of two per cent.

Inulin and para-aminohippuric acid were determined by the method outlined by Smith *et al.* (22). Creatinine was determined by the method of Folin and Wu (23).

EXPERIMENTAL RESULTS

Glomerular filtration. Table 1 shows the results obtained for the GF at ground level and at altitudes. Moderate variations are noted in the altitude values as compared to the values obtained at ground level, with the exception of *dog 3* at 18,000 feet. In this case a rather striking increase in the C_{CR} was observed. This change, which occurred upon the first exposure of this animal to altitude, was accompanied by overt symptoms of respiratory distress, cyanosis and edema of the paws. Repetition of the C_{CR} and C_{PAH} at a later date gave an average C_{CR} for four consecutive periods of 58.6 and a C_{PAH} of 164.4.

Effective renal plasma flow. The data obtained here (table 1) indicate an increase in the effective renal plasma flow in all dogs at 18,000 feet. At 24,000 feet *dog 1* showed a further increase in effective renal plasma flow while *dogs 2* and *3* showed a C_{PAH} decreased below ground level values. From this table it will be seen that no positive correlation is apparent for the changes produced in C_{CR} and C_{PAH} at altitude.

Filtration fraction (FF). Goldring and Chasis (24) have enumerated various evidences that the filtration fraction is determined primarily by the degree of patency of the efferent glomerular arteriole. If this assumption is correct then certain interpretations of the filtration fraction in the *group 1* dogs are justifiable.

The progressive reduction in the FF of *dog 1* (table 1) is probably best explained by a decrease in efferent arteriolar tonus (vasodilatation). The possibility of a pyrogenic reaction must be considered as the dog's temperature, although recorded at the start of each experiment, was not followed through the course of the urine collection periods. This would seem somewhat unlikely in view of the fact that the inulin was prepared in the same manner in all cases, the same tubing was used for each dog, and the decreases in the FF occurred on different days.

TABLE 1. EFFECTS OF ALTITUDE ON GF, C_{PAH}, FF, AND TM_{PAH}

DOG NO.	WT.	GROUND LEVEL					18,000 FEET					24,000 FEET				
		Date	GF	C_{pah}	FF	Tm_{pah}	Date	GF	C_{pah}	FF	Tm_{pah}	Date	GF	C_{pah}	FF	Tm_{pah}
	kg.															
1	8.6	3-11-47	54.5 (5)	161.0 (5)	.343 (5)	9.1 (5)	3-18-47	50.0 (4)	207.1 (4)	.259 (4)	13.1 (4)	4-11-47	67.2 (4)	382.0 (4)	.177 (4)	8.2 (4)
			55.6 (5)					37.5 (4)					68.3 (4)			
			55.0 (10)					43.8 (8)					67.8 (8)			
2	10.5	3-20-47	46.5 (5)	145.0 (5)	.321 (5)	22.6 (4)	3-27-47	44.5 (3)	177.3 (3)	.252 (3)	15.3 (4)	4- 8-47	43.1 (4)	111.5 (4)	.406 (4)	25.8 (4)
			43.0 (4)					49.5 (4)					43.4 (4)			
			44.9 (9)					47.4 (7)					43.3 (8)			
		9-24-47	53.2 (3)			27.0 (7)	9-24-47	58.1 (4)				9-24-47	53.8 (4)			
		10-13-47	39.7 (7)													
		10-23-47	46.1 (7)													
3	8.6	3- 7-47	39.3 (4)	199.2 (4)	.203 (4)	20.1 (4)	3-14-47	104.9 (5)	303.0 (5)	.278 (5)	37.7 (5)	4- 7-47	60.2 (5)	144.3 (5)	.416 (5)	65.1 (4)
			44.2 (4)					92.1 (5)					56.6 (4)			
			41.8 (8)					**98.5 (10)**					**58.6 (9)**			
							4-24-47	58.6 (4)	164.4 (4)	.356 (4)						
4	21.8	4-21-47	105.9 (5)			87.0 (4)	9-15-47	64.2 (4)			88.9 (4)	9-15-47	63.6 (3)			204.3 (3)
		5- 5-47	97.4 (5)													
		9-15-47	95.5 (4)													
		10-16-46	87.3 (4)													
		11- 3-47	100.8 (4)													
5	22.7	5-19-47	99.8 (10)			85.6 (3)	9-23-47	88.0 (4)			83.2 (4)	9-23-47	61.0 (4)			78.7 (4)
		8- 5-47	110.0 (5)			75.5 (4)										
		9-18-47	96.0 (3)													
		9-23-47	95.0 (4)													
		10-31-47	94.9 (9)													
		11- 6-47	88.0 (3)													

Figures in parentheses refer to the number of periods for which the figure quoted is the mean value.

The values in bold type are averages for the day. It will be noted that on some days there was a considerable difference between the GF values obtained during C_{PAH} periods and those obtained during Tm_{PAH} periods.

In *dog 2* the FF at 18,000 feet was somewhat decreased. The marked rise in the FF at 24,000 feet is strongly indicative of a rather marked vasoconstriction of the efferent glomerular arterioles. The FF of *dog 3* showed a progressively marked increase with an increasing altitude which we interpret as evidence of increasing efferent glomerular arteriolar vasoconstriction.

Maximum tubular excretory capacity. The data pertaining to the Tm_{PAH} values for ground level, 18,000 feet, and 24,000 feet are also shown in table 1. In 4 of the dogs at 18,000 feet and in 3 of them at 24,000 feet no significant changes in Tm_{PAH} occurred. A suggestive decrease in the Tm_{PAH} at 18,000 feet as compared to her ground level value was observed in *dog 2*. What significance, if any, should be assigned to this change, viz., Tm 22.6 to Tm 15.3 is difficult to ascertain in view of the absence of statistical data on normal Tm variations in dogs. In *dog 3* at 18,000 feet and in *dogs 3* and *4* at 24,000 feet significant increases in the Tm_{PAH} were observed. These changes were 88 per cent, 222 per cent and 135 per cent, respectively.

DISCUSSION

The data presented in this study suggest differences in the effect of altitude on C_{CR} and C_{IN} with a tendency to increase in the former and to decrease in the latter. However, these differences must be attributed to individual variation in response since other data on these same animals at altitude fail to corroborate this suggested pattern of response. Indeed, simultaneous observation of C_{CR} and C_{IN} at 18,000 feet in one of the mongrel dogs revealed a $\dfrac{C_{CR}}{C_{IN}}$ ratio of 1.01.

The effective renal plasma flow was increased in all of the animals upon whom the C_{PAH} was determined at 18,000 feet. At 24,000 feet the C_{PAH} was further increased in *dog 1*, while in the remaining 2 dogs it was below the ground level values. This suggests that in an animal exposed to increasing degrees of anoxia a point of maximum effective renal plasma flow is reached and that further increase of anoxia beyond this point results in a lowering of the effective renal plasma flow. The degree of anoxia which will elicit an increased effective renal plasma flow is of course dependent on the individual animal's tolerance and it might be reasonably expected that *dog 1* would show a similar pattern of response at a somewhat greater altitude to that shown by *dogs 2* and *3* at the altitudes studied. In severe conditions of shock and hemorrhage the renal blood flow is diminished by renal vasoconstriction making more blood available for other parts of the body. From this data it appears that a similar situation obtains in the case of severe anoxic stress. The effective renal plasma flow was greatly elevated simultaneously with the remarkably high C_{CR} in *dog 3* at 18,000 feet, but at 24,000 feet a significant rise in C_{CR} did not accompany the comparable increase in C_{PAH} in *dog 1*. Therefore, it may be said that a marked elevation in C_{PAH} produced by exposure to anoxia does not necessarily imply a concomitantly large rise in the glomerular filtration rate, nor does a decrease in C_{PAH} entail a corresponding decrease in glomerular filtration rate.

The results obtained by determination of the changes in the filtration fraction in the *group 1* dogs are interesting in that the FF values obtained at altitude differed significantly from the ground level values and in all cases the changes noted were

marked. The fact that no characteristic trend was indicated does not detract from the importance of this observation. Rather it would seem to point up the theory first enunciated by Richards and Plant (25) and later persuasively presented by Toth (8) that the release of epinephrine at varying rates can account for these changes. Although we do not believe that this explanation is necessarily the only acceptable one, we interpret these changes to indicate an alteration of glomerular dynamics by changes in the tonus of the efferent glomerular arterioles.

The whole mechanism of renal circulatory adjustments to conditions of stress seems to be set to insure an effective glomerular filtration rate. If this is the case, the changing FF is a convenient numerical index of the progress of these adjustments. The effective renal plasma flow and the glomerular filtration rate are indices of the effectiveness of this mechanism; the former of its service to the circulation as a whole, the latter of its effectiveness in maintaining kidney function.

The decrease of the available oxygen to the kidney tubules produced in these studies does not result in a noticeably decreased maximum tubular excretory ability. Indeed, the increases in Tm_{PAH} observed in 3 of the 5 dogs point to an apparent increase in the ability of the tubule cells to excrete PAH upon exposure of the animal to an atmosphere of sufficiently reduced oxygen tension. These observations agree with those of Alving *et al.* (1) in the case of chronic, intermittent exposure to anoxia.

An increase in the maximum tubular excretory ability has been effected by the administration of anterior pituitary lobe extract and thyroid hormone to normal and hypophysectomized dogs (26). Eiler *et al.* (27) produced tremendous increases in Tm_D by administration of thyroxin. Using testosterone propionate Welsh *et al.* have been able to increase the Tm_D in dogs up to 100 per cent (28). These studies indicate that hormonal influences are capable of causing alterations in the tubular transfer mechanism of the kidney. The possibility of a hormonal factor being instrumental in causing the elevation of the Tm_{PAH} in the dogs used in this study must be considered.

The two mechanisms by which an increase in Tm_{PAH} could be effected are *a*) an increase in the number of participating nephrons and *b*) an actual increase in the ability of the tubular cells to transfer PAH. If the former were the case, the ratio $\dfrac{GF}{Tm_{PAH}}$ would remain unchanged; if the latter were the case, this ratio would be reduced. It will be noted from table 1 that in *dog 3* at 18,000 feet both the C_{IN} and Tm_{PAH} are increased to approximately twice the ground level values and therefore the ratio $\dfrac{GF}{Tm_{PAH}}$ remains roughly constant. However, in the other instances in which an elevated Tm_{PAH} was observed no corresponding increase in GF occurred, and therefore this ratio decreased markedly. Thus it can be stated that in two of the three experiments in which an increased Tm_{PAH} was observed this increase is attributable to an increased ability of the tubular cells to transfer PAH, whereas in the third case an increase in the number of participating nephrons is implicated. In this regard, it is interesting that Alving *et al.* (2) found their increases in Tm_D in the absence of changes in GF, thereby implicating an increase in the ability of the tubular cells to secrete rather than an increase in the number of participating nephrons.

SUMMARY

Five dogs were subjected to renal function studies at ground level and at simulated altitudes of 18,000 feet and of 24,000 feet. In all cases C_{PAH} determinations were started five minutes after reaching the desired altitude. In the case of the *group 2* animals Tm_{PAH} measurements were likewise started five minutes after attaining the desired altitude, while in the case of the *group 1* animals the Tm_{PAH} measurements were started approximately 75 minutes after attaining the desired altitude. The glomerular filtration rate in these animals was either increased, decreased or unaffected depending upon the reaction of the individual animal to reduced ambient pressure. The effective renal plasma flow was increased in all dogs at an altitude of 18,000 feet and was further increased in one dog but decreased below the ground level values in the remaining dogs at 24,000 feet. The maximum tubular excretory ability was markedly increased at 18,000 feet in 1 animal and at 24,000 feet in 2 of the 5 animals studied.

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FURTHER OBSERVATIONS ON THE EFFECTS OF ALTITUDE ANOXIA ON RENAL FUNCTION

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IN A previous communication (1) we have reported the results of investigations conducted in our laboratory on the effects of altitude anoxia on clearances of inulin, creatinine, and para-aminohippuric acid and on the maximum tubular excretory capacity for para-aminohippuric acid. In these investigations the glomerular filtration rate was either increased, decreased or unaffected depending upon the reaction of the individual animal to exposure to reduced barometric pressure; the effective renal plasma flow as determined by C_{PAH} was increased at an altitude of 18,000 feet in all dogs and was further increased in one dog but decreased below the ground level values in the remaining 2 dogs at 24,000 feet. The maximum tubular excretory ability was markedly increased in one dog at 18,000 feet and at 24,000 feet in 2 of the 5 animals studied.

The present studies constitute an extension of these investigations to include the effects of altitude anoxia on the maximum rate of tubular reabsorption of glucose (Tm_G) and on the simultaneous Tm_G and Tm_{PAH}.

The concept of a maximum rate of tubular reabsorption of glucose was established by Shannon and Fisher (2) who demonstrated that at high levels of plasma glucose a definite and constant amount of glucose in mg/min. is reabsorbed by the tubules irrespective of variations of the plasma glucose levels above the minimum level required to saturate the reabsorptive mechanism.

Shannon (3) states that "relatively few precautions need be followed for valid measurements of glucose Tm provided the animal is well hydrated and the arterial plasma glucose is maintained at an adequate and fairly constant value. Under these conditions the system in the dog has surprising stability, and the glucose Tm of any animal is quite constant over a period of many months. Excessive insulin may acutely depress it, but it is not affected by epinephrine nor by marked changes in dietary regime, and it is not related to the concurrent rate of glomerular filtration." It has been recently demonstrated that the administration of thyroxin greatly increases the Tm_G (4).

Klopp, Young, and Taylor (5) have found that high plasma levels of glucose may decrease the Tm_{PAH}, that high plasma levels of PAH may increase the Tm_G, and that high plasma levels of both simultaneously may increase the glomerular filtration rate. Houck (6) has reported that Tm_G and Tm_{PAH} are both depressed when measured simultaneously with Tm_{PAH} depressed approximately twice as much as Tm_G. The magnitude of the changes observed was not great in either case in this study.

EXPERIMENTAL PROCEDURE

Three well trained, adult female dogs, one mongrel and 2 Dalmatian coach hounds, were used in these studies. The dogs were unanesthetized and loosely re-

.strained on a comfortable animal board. The technique used in obtaining blood and urine specimens and insuring complete evacuation of the bladder was the same as previously outlined (1). The renal function tests were performed in an altitude chamber at ground level and at simulated altitudes of 18,000, 24,000, and 28,000 feet (one test).

The procedure followed in each case in studying the effect of altitude anoxia on Tm_G was as follows: after the dog was placed in the altitude chamber on the animal board, a primer dose of glucose and inulin was given intravenously and immediately a constant infusion of glucose and inulin was started. (In the case of the mongrel, *dog 3*, creatinine was used rather than inulin.) At least 20 minutes later the first urine collection period was begun. Several periods were run at ground level. The pressure was then decreased to a simulated altitude of 18,000 feet at a rate of ascent of 3000 feet per minute, and after approximately 10 minutes the first period at this altitude was commenced. Upon the completion of several periods further ascent to a simulated altitude of 24,000 feet was accomplished at the same rate of ascent and after an interval of 10 minutes several more periods were carried out at this altitude.

In the studies of simultaneous Tm_G and Tm_{PAH} the primer and infusion solutions contained sodium para-aminohippurate in addition to the materials mentioned above. The procedure in these studies was the same as above with the exception that no studies were made at 18,000 feet, but following the periods at ground level the dogs were taken directly to an altitude of 24,000 feet at the rate of 3000 ft/min. In one case (*dog 2*) at the completion of four periods at 24,000 feet the dog was taken to an altitude of 28,000 feet and three more periods were carried out.

Inulin and para-aminohippuric acid were determined by the methods outlined by Smith (7). Creatinine was determined by the method of Folin and Wu (8). Glucose was determined by the Folin method (9). All measurements were made on an Evelyn photoelectric colorimeter.

EXPERIMENTAL RESULTS

The data obtained regarding the effect of exposure of the animals to altitude anoxia on their maximum tubular reabsorptive ability are shown in table 1. It will be noted that at 18,000 feet there was in one animal a relatively small decrease in Tm_G of approximately 20 per cent, in one animal a larger decrease in Tm_G of approximately 55 per cent, and in one animal no change in Tm_G in two separate sets of experiments. It is interesting to note that although there were quite definite changes in Tm_G at 18,000 feet as compared to the ground level values, there were no appreciable differences observed in any of the animals between the values obtained at 18,000 feet and those obtained at 24,000 feet. It is also interesting that in no case was there any marked change in the glomerular filtration rate at altitude as compared to the ground level values.

The data obtained in the studies of simultaneously determined Tm_G and Tm_{PAH} are shown in table 2. In those studies conducted under conditions of normal atmospheric pressure (ground level) a distinct depression of the Tm_{PAH}, as compared to the Tm_{PAH} of the animals when determined in the absence of high glucose plasma levels, was observed in all animals. These observations are in agreement with the previous reports of Klopp *et al.* (5) and of Houck (6). Repetition of these experiments at

24,000 feet produced no significant changes in the values of Tm_{PAH} found. In other words, when Tm_{PAH} and Tm_G were simultaneously determined there resulted a marked depression of the dog's normal Tm_{PAH} whether or not the animal was being exposed to reduced barometric pressure; the extent to which the Tm_{PAH} was depressed did not seem to be related to the percentage of atmospheric oxygen to which the animal was being subjected.

The values of Tm_G obtained when Tm_G and Tm_{PAH} were simultaneously determined at ground level were in two cases (*dogs 2 and 3*) in good agreement with the values obtained when the Tm_G was determined in the absence of high plasma levels

TABLE 1. EFFECT OF ALTITUDE ON Tm_G

DOG NO.	WT.	DATE	GROUND LEVEL		18,000 FEET		24,000 FEET	
			GF	Tm_G	GF	Tm_G	GF	Tm_G
	kg.							
1	22.7	10– 8-47	85.2 (3)	394.3 (3)	86.0 (3)	316.0 (3)	92.5 (4)	319.6 (4)
2	21.8	9–29-47	94.9 (4)	352.3 (4)	88.5 (4)	164.7 (4)	106.2 (4)	155.9 (4)
3	10.5	10– 1-47	42.8 (4)	157.9 (4)	44.3 (4)	126.6 (4)	46.4 (4)	143.7 (4)
		10– 9-47	48.1 (4)	134.9 (4)	42.8 (4)	137.5 (4)	47.2 (4)	126.5 (4)

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

TABLE 2. EFFECT OF ALTITUDE ON SIMULTANEOUSLY DETERMINED Tm_G AND Tm_{PAH}

DOG NO.	WT.	DATE	GROUND LEVEL			24,000 FEET			28,000 FEET		
			GF	Tm_G	Tm_{PAH}	GF	Tm_G	Tm_{PAH}	GF	Tm_G	Tm_{PAH}
	kg.										
1	22.7	10-14-47	107.1 (4)	236.7 (4)	10.9 (4)	108.3 (4)	205.9 (4)	7.3 (4)			
		10-22-47	61.9 (4)	187.9 (4)	25.1 (4)	67.2 (4)	168.0 (4)	30.0 (4)			
2	21.8	10-10-47	59.9 (6)	333.5 (6)	37.8 (6)	106.1 (4)	256.8 (4)	21.2 (4)	83.9 (3)	182.6 (3)	24.5 (3)
3	10.5	10-29-47	39.8 (4)	151.2 (4)	0.3 (4)	42.5 (4)	147.7 (4)	—2.3 (4)[1]			

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

[1] Range for 4 periods was—0.6 to—5.2. Negative values for Tm_{PAH} have recently been reported (11). We have also noted an occasional negative Tm_{PAH} in other experiments.

of PAH; however, in the case of *dog 1*, in two separate sets of experiments at ground level, a rather marked decrease in Tm_G (40 per cent on one occasion and 52 per cent on one occasion) as compared to the values obtained in the absence of high plasma levels of PAH was noted. This observation is in disagreement with the previous report of Klopp *et al.* (5) that Tm_G is either unaffected or increased by the presence of high plasma PAH levels but in agreement with the findings of Houck (6).

Comparison of the Tm_G values obtained at ground level and at 24,000 feet reveals that in the presence of high plasma levels of PAH the tendency toward decreasing Tm_G with increasing altitude is still apparent, as was the case in the absence of high plasma levels of PAH, but this tendency is considerably less marked. For example, in the case of *dog 2* in the absence of high plasma levels of PAH the Tm_G was reduced to 47

per cent of the ground level value at 18,000 feet and to 44 per cent of the ground level value at 24,000 feet, whereas in the presence of high plasma levels of PAH it was reduced only to 77 per cent of the ground level value at 24,000 feet and even at 28,000 feet only to 55 per cent of the ground level value.

Table 3 summarizes the data we have obtained showing the effect of altitude anoxia on Tm_G both in the presence and in the absence of high plasma levels of PAH and its effect on Tm_{PAH} both in the presence and in the absence of high plasma levels of glucose. The data shown in this table on the effect of altitude on Tm_{PAH} in the

TABLE 3. Tm_G AND Tm_{PAH} DETERMINED INDIVIDUALLY AND SIMULTANEOUSLY AT VARIOUS ALTITUDES

DOG NO.	WT.	DATE	DETERMI-NATION	GROUND LEVEL Individually	GROUND LEVEL Simul-taneously	18,000 FT. Individually	24,000 FT. Individually	24,000 FT. Simul-taneously
	kg.							
1	22.7	10– 8–47	Tm_G	394.3 (3)		316.0 (3)	319.6 (4)	
		10–14–47	Tm_G		236.7 (4)			205.9 (4)
		10–22–47	Tm_G		187.9 (4)			168.0 (4)
		9–18–47	Tm_{PAH}	85.6 (3)				
		9–23–47	Tm_{PAH}	75.5 (4)		83.2 (4)	78.7 (4)	
		10–14–47	Tm_{PAH}		10.9 (4)			7.3 (4)
		10–22–47	Tm_{PAH}		25.1 (4)			30.0 (4)
2	21.8	9–29–47	Tm_G	352.3 (4)		164.7 (4)	155.9 (4)	
		10–10–47	Tm_G		333.5 (6)			256.8 (4)
		9–15–47	Tm_{PAH}	87.0 (4)		88.9 (4)	204.3 (3)	
		10–10–47	Tm_{PAH}		37.8 (6)			21.2 (4)
3	10.5	10– 1–47	Tm_G	157.9 (4)		126.6 (4)	143.7 (4)	
		10– 9–47	Tm_G	134.9 (4)		137.5 (4)	126.5 (4)	
		10–29–47	Tm_G		151.2 (4)			147.7 (4)
		3–20–47	Tm_{PAH}	22.6 (4)				
		3–27–47	Tm_{PAH}			15.3 (4)		
		4– 8–47	Tm_{PAH}				25.8 (4)	
		10–13–47	Tm_{PAH}	27.0 (7)				
		10–29–47	Tm_{PAH}		0.3 (4)			−2.3 (4)

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

absence of high plasma levels of glucose are taken from a previous publication (1) and are introduced at this point merely for purposes of comparison. It will be noted from this table, in addition to the points previously stressed, that in *dog 2* the immense increase of Tm_{PAH} at 24,000 feet as compared to the ground level value reported in our previous paper is not apparent in the present studies where Tm_G and Tm_{PAH} are simultaneously determined. It is not possible to state whether the failure of this interesting finding to recur is attributable to the high plasma levels of glucose in the present experiments. We feel that it could conceivably be ascribed to the high plasma levels of glucose, not on the basis of competition between PAH and glucose with regard to the tubular transfer mechanism but merely on the basis of the improved altitude

tolerance commonly known to be mediated by the administration of glucose. We have tried on numerous occasions to duplicate results of one day's experiment at altitude on another day, both with regard to renal function studies and with regard to other investigations in which we have been interested, and have found this to be a virtual impossibility if the same criteria of satisfactory reproduction of results are employed as those invoked at ground level. The variations of results at altitude from day to day are considerably greater than those observed under conditions of normal atmospheric pressure although, in general, the results are qualitatively and within a certain degree of accuracy quantitatively reproducible. This fact may be attributed to any or all of several factors (10), but it must be borne in mind that a given animal's tolerance to anoxic exposure varies from day to day. It has seemed to us that the most pronounced changes in renal function have been observed on the days when the dogs have appeared to be the most distressed by exposure to altitude. Therefore, it seems possible that the high plasma glucose level may have been important in preventing the occurrence of elevation of the Tm_{PAH} value at altitude during the experiment in which Tm_{PAH} and Tm_G were simultaneously determined. However, one cannot be certain that this phenomenon would have recurred even in the absence of a high plasma level of glucose.

Examination of the values obtained for glomerular filtration rates in the experiments in which Tm_G and Tm_{PAH} were simultaneously determined reveals some interesting changes that occurred in this regard. In the case of *dog 1* on one experimental day a GF of 107 was observed and on another day a GF of 62 was found. In neither case was this value appreciably altered upon exposure to altitude. This dog's normal GF has been established by numerous determinations (34 periods) to average 98.1 with a range of from 88 to 110. In the case of *dog 2* a GF of 60 was observed at ground level. This value was increased to 106 at 24,000 feet and decreased to 84 at 28,000 feet. This dog's normal GF has been established (22 periods) to average 97.7 with a range of 87.3 to 105.9. Thus in these two cases the C_{IN} was definitely decreased below the normal values for these dogs. In this respect we again find our results at variance with those of Klopp *et al.* who found an increase of glomerular filtration rate over the normal value during their experiments in which Tm_G and Tm_{PAH} were simultaneously determined.

SUMMARY

Three dogs were submitted to renal function tests at ground level and at simulated altitudes in an altitude chamber. The tests performed were determination of Tm_G alone and simultaneous determination of Tm_G and Tm_{PAH}. In 2 of the 3 animals a decrease in Tm_G was observed at 18,000 feet as compared to the ground level values, but in the 3rd animal no change was evident. In none of the animals was there any appreciable difference between the value obtained at 18,000 feet and the value obtained at 24,000 feet.

When Tm_{PAH} and Tm_G were simultaneously determined there resulted a marked depression of the dog's normal Tm_{PAH} whether or not the animal was being exposed to reduced barometric pressure, and the extent to which the Tm_{PAH} was depressed bore no evident relationship to the altitude to which the animal was being subjected.

In one animal a marked derpression of the Tm_G resulted when Tm_G and Tm_{PAH} were simultaneously determined at ground level, but in the other 2 animals no such depression was noted.   At high plasma levels of PAH the tendency toward decreasing Tm_G with increasing altitude, as in the case of Tm_G determinations in the absence of high plasma levels of PAH, was still apparent but less pronounced.   In 2 of the 3 dogs at ground level, the glomerular filtration rate was distinctly depressed in the presence of high plasma levels of PAH and of glucose simultaneously.   In one of these dogs exposure of the animal to altitude caused the GF to return to normal, but in the other case it did not do so.

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EFFECTS OF ALTERATIONS IN BODY TEMPERATURE ON PROPERTIES OF CONVULSIVE SEIZURES IN RATS[1,2]

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IT IS well known that convulsive seizures may frequently be associated with acute febrile illnesses in early childhood, but less frequently in adult life (1, 2). Convulsions have also been reported to occur in patients subjected to fever therapy (3–5). In some epileptic subjects the frequency of seizures may be increased during fever, while in others the attack rate may be diminished (2). Experimentally, acute hyperpyrexia has been shown by Wegman (6) to cause convulsions in kittens. With regard to low body temperature, human refrigeration has been reported by Fay (7) to increase the excitability of the deep reflexes. Similarly, Barron and Matthews (8), and Ozorio De Almeida (9, 10) have shown that a reduction in body temperature of frogs increases the excitability of the spinal cord. In peripheral nerve Granit and Skoglund (11) have observed facilitation of ephaptic transmission, and Lorente de Nó (12) has shown that the rheobase is decreased when temperature is reduced.

Because of the paradoxical occurrence of febrile convulsions in some patients in contrast to febrile remission of seizures in others, it seemed important to analyze the effect of alterations in body temperature on various properties of experimental seizures without the complications, such as infection, dehydration, etc., which may attend febrile illness.

METHODS

Body temperature of Sprague-Dawley rats was altered by restraining the animals in circular wire mesh holders either in a refrigerator at a temperature of −8°C. or in an insulated heating cabinet at 55°C. until the desired rectal temperature was obtained. Rectal temperatures were determined with a mercury thermometer immediately before and after experimental seizures.

Electroshock seizures were produced by a 60-cycle alternating current apparatus designed by Dr. Lowell A. Woodbury; the current delivered is independent of the external resistance. Shocks were of 0.2 second duration, and were delivered through corneal electrodes. Minimal electroshock seizure thresholds (13) were compared at control and experimental body temperatures, with a period of at least 12 hours between tests for each animal. For observation of changes in pattern and duration of

maximal seizures (14), supramaximal shocks of 150 mA. (or five times threshold) were given; the interval between tests was at least two hours.

To determine the effect of altered temperature on rate of recovery of maximal seizure pattern, groups of 4 to 12 rats were used at each desired body temperature. All were given a conditioning supramaximal shock, followed by a supramaximal test shock after the desired interval. The percentage of animals showing full recovery of seizure pattern, including the tonic extensor component, was noted. Percentage recoveries for various intervals were plotted on probit paper as a function of time, and
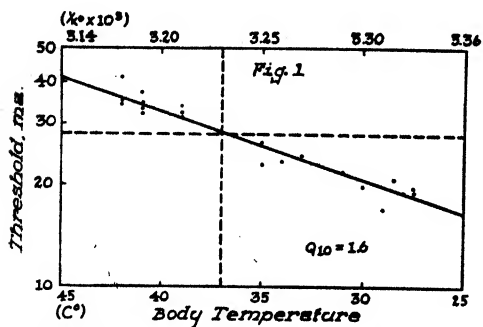

Fig. 1. EFFECT OF BODY TEMPERATURE on the threshold for minimal electroshock seizures in rats. Electroshock threshold in mA. is plotted on a logarithmic scale (ordinate) as a function of the reciprocal of the absolute temperature (*abscissa, top*). For ease in interpretation the corresponding centigrade degrees are also shown (*abscissa, bottom*). *Vertical broken line:* normal body temperature (average of 21 rats); *horizontal broken line:* normal electroshock threshold (average of 159 rats). Each point along solid diagonal line represents one experiment.
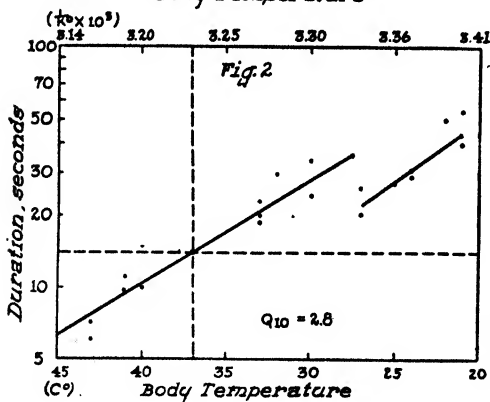
Fig. 2. EFFECT OF BODY TEMPERATURE on the duration of maximal electroshock seizures in rats. Electroshock threshold in mA. is plotted on a logarithmic scale (ordinate) as a function of the reciprocal of the absolute temperature (*abscissa, top*). For ease in interpretation the corresponding centigrade degrees are also shown (*abscissa, bottom*). *Vertical broken line:* normal body temperature (average of 22 rats); *horizontal broken line:* total duration of maximal seizures at normal body temperature (average of 73 rats). Each point along solid diagonal line represents one experiment.

the resulting points fitted by eye to determine graphically the time for recovery of full seizure pattern in 50 per cent of the animals at each body temperature.

For determination of the rate of recovery of minimal seizure threshold, the method was the same except that an arbitrary value of 150 per cent of the unconditioned threshold at the same temperature was selected for the test shock.

Chemoshock seizure thresholds for intraperitoneally injected Metrazol or picrotoxin were determined by treating groups of four or more animals at each of several dose levels at each desired temperature and finding graphically (as above) that quantity of drug which would convulse 50 per cent of the animals at each temperature.

RESULTS

The minimal electroshock seizure threshold was found to vary directly with body temperature. When the logarithm of threshold was plotted against the reciprocal

of the absolute temperature (fig. 1.), the data were approximately fitted by a straight line giving a μ value of 7500 calories per mole, corresponding to a Q_{10} of 1.6.

The susceptibility to convulsions induced by picrotoxin or Metrazol was also increased by lowering the body temperature. The approximate Q_{10} for picrotoxin was found to be 1.6, which is in agreement with the electroshock data. Occasional spontaneous seizures were observed when body temperature was elevated above 43°C. or reduced below 27°C. This would tend to compromise any observations taken beyond these limits.

The effect of body temperature on the total duration of maximal seizures is shown in figure 2. As might be expected the total duration varies inversely with the body temperature. The Q_{10} was found to be 2.8 (μ value, 19,600 calories per mole). It is interesting to note that a sharp reduction in total seizure duration occurs when body temperature is reduced below 27°C., as shown by the separate line in the upper right segment of figure 2. It should be mentioned that spontaneous convulsions were occasionally observed at and below this temperature.

Fig. 3. EFFECT OF BODY TEMPERATURE on time for recovery of maximal electroshock seizure pattern in rats. Each point (x, open circle or solid circle) on the graph represents a group of 4 to 12 rats. The percentage recoveries (*left ordinate;* corresponding probit values, *right ordinate*) are plotted as a function of time (*abscissa*) and the points fitted by eye.
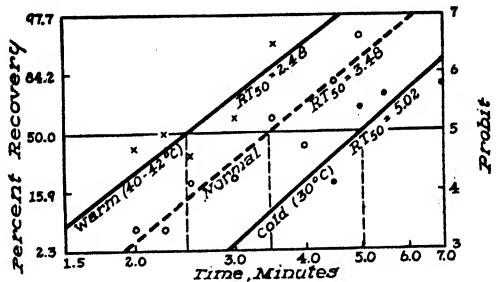
Associated with the effect of variations in temperature on total seizure duration, changes in the relative duration of the various seizure components were observed. In general, a reduction in temperature decreased the fractional duration of the tonic phase and particularly the initial flexor component of the tonic phase. At elevated body temperatures the absolute as well as the relative duration of tonic flexion was increased; above 42°C. the entire seizure tended to be a tonic flexion with superimposed fine clonus. It was difficult to measure the duration of the various components at temperatures below 27°C. because the end-points were not clearly defined.

The effect of body temperature on the time for recovery of a full maximal seizure pattern following a supramaximal shock is shown in figure 3. It may be seen that the recovery time for 50 per cent of normal rats is 3.48 ± 0.25 minutes. When the body temperature was elevated to 40° to 42°C. the recovery time for 50 per cent of the animals was found to be 2.48 ± 0.25 minutes. Conversely when body temperature was reduced to 30°C. it required 5.02 ± 0.44 minutes for 50 per cent of the animals to recover the maximal seizure pattern. Therefore the Q_{10} between 30°C. and 40°C. is approximately 2 (μ value of 12,000 calories per mole) for recovery of the normal seizure pattern.

Following a maximal seizure, the rate of recovery of threshold was found to be doubled by a 10° reduction in body temperature, giving a Q_{10} of 2. This is in agreement with the results of the maximal shock experiments.

DISCUSSION

It is of interest to note that the temperature coefficients differ for seizure threshold, seizure duration and post-seizure recovery.   This would seem to indicate a difference in the fundamental chemical processes underlying these three functions (15), but the identification of the specific temperature coefficients with particular limiting enzyme steps would be unwarranted (16).

Since seizure threshold was increased by a rise in body temperature, this factor alone might conceivably account for febrile remission of convulsive disorders in some patients.   It obviously could not account for febrile onset of seizures in other cases. Spontaneous seizures were occasionally seen at high body temperatures in the present study, demonstrating that threshold is not the only factor determining the occurrence of convulsions.

SUMMARY

Characteristics of experimental seizures were studied in rats whose body temperatures were altered by exposure to extreme environmental temperatures.   Seizure threshold was increased, seizure duration reduced and post-seizure recovery hastened by increased body temperature, and conversely changed by decreased body temperature.   The data were adequately fitted by plotting the logarithm of each function against the reciprocal of absolute body temperature.   For seizure threshold the Q_{10} was found to be 1.6, for seizure duration 2.8 and for recovery 2.0.   The possible significance of the findings is briefly discussed.

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EXCRETION OF BILIRUBIN AND BROMSULFALEIN IN BILE

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IN CONTRAST to the abundance of information regarding the rate of disappearance from the blood of intravenously injected bilirubin and bromsulfalein, relatively little is known concerning the rate of their excretion in the bile. Practically all of the few reported data bearing on this problem have been obtained under conditions that are not entirely satisfactory for one or more of the following reasons: *a*) the effect of anesthesia and reaction to operation in acute experiments; *b*) the inability to maintain consistently normal nutrition and liver function in the usual external bile-fistula dog; *c*) the use of inaccurate methods for determination of pigment in bile.

The most important difficulty has been overcome by the use of the Thomas-type tubulated duodenal-fistula dog (1-3), in which normal nutrition and liver function can be maintained for periods of many months to several years. Moreover, accurate methods are now available for determination of total pigment and bromsulfalein in bile. The present study was undertaken for the purpose of securing information regarding the normal rate of excretion of pigment in the bile and the rate of excretion of pigment and bromsulfalein after intravenous injection of bilirubin and bromsulfalein, singly and in combination. These experiments are to serve as controls for a subsequent study of the influence of various choleretic agents upon the biliary excretion of these substances.

Materials and Methods

Ten trained, cholecystectomized dogs were used, provided with gastric and duodenal fistulae fitted with large cannulae, as described by Thomas (1). The duodenal fistula was placed opposite the ampulla of Vater and bile was collected by inserting a temporary glass cannula (2, 3) into the common duct. The bile was allowed to drain into graduated tubes until the flow became constant. It was then collected in 15-minute samples.

In control studies, collections were continued for periods ranging from 2 to 4 hours. In experiments involving intravenous injection of bilirubin or bromsulfalein, bile was collected for 30 to 60 minutes before injection and for varying periods up to 6 hours subsequently. In experiments involving continuous injection, these were made with a motor-driven constant infusion apparatus, at measured rates, after injecting a priming dose. Bilirubin (Eastman Kodak Co.) was injected in one per cent Na_2CO_3 solution (0.3-2.0 mg/cc.). In continuous injection experiments, the commercial bromsulfalein solution was diluted with 0.85 N NaCl solution. The amounts injected are indicated in the tables.

All determinations of pigment and dye were made with the Evelyn photoelectric colorimeter. Each 15-minute bile sample was made up to 5 or 10 cc. with distilled water. Total bile pigment was determined by the method of Malloy (4), bromsulfalein in bile, by a method described by Cantarow

and Wirts (5); bilirubin in serum, by the method of Malloy and Evelyn (6); and bromsulfalein in blood, by the method of Bradley *et al.* (7).

RESULTS AND COMMENT

Controls. Data regarding bile volume and pigment output in 8 untreated dogs during 44 periods of at least one hour are presented in table 1. The bile flow ranged from 2.1 to 17.6 cc/hr., 0.140 to 1.257 cc/kg/hr. The bile pigment concentration ranged from 12 to 161 mg/100 cc., and the output from 1.0 to 5.943 mg/hr., 0.019 to 0.289 mg/kg/hr. Both volume and pigment varied considerably in individual animals. For example, the findings in *dog 6*, studied on 16 different days, were as follows: bile volume, 2.1 to 15.2 cc/hr. (0.105–0.760 cc/kg/hr.); pigment concentration, 23 to 153 mg/100 cc.; pigment output, 1.870 to 4.281 mg/hr. (0.093–0.214 mg/kg/hr.).

Early in this study it was found that the presence of food in the stomach was accompanied by wide fluctuations in bile output, pigment concentration and, occasionally, total pigment output in 15-minute periods. This was attributed to the

TABLE 1.   BILE VOLUME AND PIGMENT EXCRETION IN UNTREATED DOGS

DOG	NO. OF DETERMI- NATIONS	VOLUME		PIGMENT		
		cc/hr.	*cc/kg/hr.*	*mg. %*	*mg/hr.*	*mg/kg/hr.*
3	1	6.0	0.283	60	3.63	0.172
4	6	3.1–13.7	0.206–0.913	12– 92	1.372–3.220	0.091–0.214
5	4	3.7– 8.1	0.205–0.450	32– 77	2.614–4.810	0.145–0.267
6	16	2.1–15.2	0.105–0.760	23–153	1.870–4.281	0.093–0.214
7	1	7.4	0.616	47	3.474	0.289
8	3	4.6– 6.5	0.383–0.541	21– 57	1.333–2.600	0.111–0.216
9	12	3.3–17.6	0.235–1.257	17–154	1.000–5.943	0.071–0.218
10	1	2.1	0.140	161	3.38	0.225

passage of acid gastric contents into the duodenum, with consequent stimulation of the secretin mechanism. Instillation of HCl into the duodenum had a similar effect. All subsequent observations were made after a 14 to 16-hour fast, which diminished but did not eliminate these fluctuations. However, the hourly flow and pigment output were quite constant under these circumstances in individual experiments. No satisfactory explanation can be offered for the variation in bile flow and pigment output on different days. This observation is not in accord with that of Kocour and Ivy (8), who reported that in external fistula dogs the bile volume output is constant (±4 per cent) and reproducible under similar experimental conditions. It may be significant in this connection that the Rous-McMaster type fistula dog (9), which they used, has been found to have, almost invariably, some degree of impairment of liver function even though apparently healthy (10, 3). Single or continuous intravenous injections of 0.85 per cent NaCl or one per cent Na_2CO_3 solutions, in quantities employed for injection of bilirubin or bromsulfalein, had no significant effect on bile volume or pigment excretion.

Bilirubin injection. Four dogs were given eight single intravenous injections of one mg. bilirubin per kg. body weight (in one minute). The pertinent data are

presented in table 2. After a single priming dose (0.3–1.0 mg/kg.), bilirubin was injected continuously for 30 to 120 minutes in 3 dogs (6 experiments).

The injection produced no significant change in the rate of bile flow. The maximum concentration of pigment in the bile ranged from 268 to 433 mg/100 cc. after single injection and from 251 to 727 mg/100 cc. after prolonged injection, occurring in the second 15-minute bile sample in one instance, in the third sample in five and in the fourth sample in eight instances. The period of maximum pigment output coincided with that of maximum concentration in all but five instances, occurring in the preceding 15-minute period in two and in the succeeding 15-minute period in three instances. No significant amount of bilirubin was found in the urine obtained by catheterization at the end of the experimental period. These data do not represent the actual conditions of excretion of pigment by the hepatic cells

TABLE 2. PIGMENT EXCRETION IN BILE FOLLOWING SINGLE INTRAVENOUS INJECTION OF 1 MG. BILIRUBIN PER KILOGRAM BODY WEIGHT

DOG	BILIRUBIN INJECTED	POST-INJECTION MAXIMUM CONCENTRATION (15 MIN. PERIOD)				PIGMENT EXCRETION				RECOVERY			
						Control	Post-injection						
		mg.%	total	volume	time		1st hr.	2nd hr.	3rd hr.	1st hr.	2d hr.	3d hr.	total
	mg.		*mg.*	*cc.*	*min.*	*mg/hr.*	*mg.*	*mg.*	*mg.*	*%*	*%*	*%*	*%*
4	14.8	320	1.60	0.5	60	2.411	6.658	7.397	3.218	28.7	33.7	5.4	67.8
	14.8	268	4.29	1.6	45	2.416	9.074	6.777	3.385	44.0	29.5	6.5	89.0
6	20.0	410	3.28	0.8	60	3.845	10.406	9.772	3.625	32.8	29.6		62.4
	20.0	343	6.52	1.9	30	4.279	15.192	7.279		54.6	15		69.6
8	12.0	334	5.00	1.5	45	1.347	9.145	2.621	2.799	65.0	10.6	12.1	87.7
	12.0	433	1.95	0.45	60	1.333	4.645	5.884	1.690	27.6	37.9	3.0	68.5
	12.0	271	4.07	1.5	60	2.600	6.880	3.393	3.398	35.7	6.6	6.6	48.9
9	14.0	317	3.49	1.1	45	3.778	7.669	7.166	4.901	27.8	24.2	8.0	60.0

because of the relatively low 15-minute bile volume (< 2 cc.) as compared to the capacity of the bile duct system. The latter was found to be about 5 cc. in one cholecystectomized, Thomas-type fistula dog terminated six months after operation. The data obtained therefore represent the result of some degree of dilution of freshly secreted bile by the contents of the duct system; there is also a time lag incident to the existence of this 'dead space'.

The percentage recovery of the injected bilirubin was calculated by subtracting the hourly pigment excretion during the pre-injection control period from the subsequent hourly excretion. The accuracy of these recovery values depends upon the validity of the assumption that the basal pigment excretion remains constant during the experimental period. Although this assumption is open to some question, the comparative consistency of hourly pigment excretion in control experiments suggests that this method of calculation is justifiable.

After a single injection of one mg/kg., 48.9 to 89.0 per cent of the amount

administered was recovered in three hours, the largest proportion during the first hour in six instances and during the second hour in two instances (table 2). In the continuous injection experiments (1.3–5.0 mg/kg.), 61.0 to 100 per cent of the quantity administered was recovered within two to four hours. There was no consistent relationship between the percentage recovered and the quantity administered. Some increase in pigment concentration and/or output was almost invariably present in the first 15-minute bile sample and was pronounced in the second 15-minute sample. Interpreted in the light of conditions incident to the existence of the relatively considerable dead space of the bile ducts, these data indicate that an increase in circulating bilirubin results in a prompt increase in the rate of pigment excretion by the hepatic cells.

As pointed out by Greene and Snell (11), this augmented excretion is accomplished primarily by an increase in concentration of pigment in the bile rather than by an increase in bile volume. However, they found no increase in bilirubin excretion during the first 30 minutes after intravenous injection of one mg/kg., the maximum concentration and excretion occurring during the third hour. This discrepancy may be due to the fact that their experiment was conducted under amytal anesthesia, the rate of bile flow being considerably lower than in our dogs. Berman, Snapp and Ivy (12) determined the rate of biliary excretion of bilirubin after injecting 5 mg/kg. in 5 dogs under sodium pentobarbital anesthesia. An average of 29 per cent of the injected bilirubin was recovered in 1.5 hours and 39.4 per cent in 3 hours, the maximum recovery occurring during the second half-hour period. It is doubtful that the greater recovery in our experiments was due to the smaller quantity administered as a single injection (1 mg/kg), inasmuch as approximately the same proportion was recovered by us after prolonged injection of larger amounts. Moreover, Berman *et al.* (12) obtained substantially the same percentage recovery after injection of 12 mg/kg. as after 5 mg/kg. It appears more probable that the lower figures obtained by them are due to the effect of the anesthetic and operative procedure and, possibly, to the fact that we determined the total bile pigment output whereas they determined only bilirubin, by the method of Thannhauser and Andersen (13), which does not give entirely reliable results for pigment in bile.

Bromsulfalein injection. Five dogs were given 11 single intravenous injections of 5 mg. of bromsulfalein (BSP) per kg. in one minute. The pertinent data are presented in table 3. After a single priming dose (1 mg/kg.), BSP was injected continuously for two hours in 4 dogs (6 experiments).

There was no significant change in the rate of bile flow. The maximum concentration of dye in the bile ranged from 292 to 1432 mg/100 cc. after single injection and from 278 to 1420 mg/100 cc. after prolonged injection, occurring in the second 15-minute bile sample in four instances in the third sample in five, in the fourth sample in four, in the fifth sample in two, in the sixth sample in two and as late as the seventh sample (105 minutes) in one. It is interesting that in this case (*dog 5*, table 3), the maximum dye output occurred during the second 15-minute period. There was no such close time correspondence between maximum output and maximum concentration of BSP as existed in the case of bile pigment.

The percentage recovery of BSP was of the same order of magnitude as that of

injected bilirubin, viz., 60 to 96.9 per cent in 3 hours after single injection and 48 to 74.7 per cent within 4 hours after beginning a continuous two-hour injection. Dye appeared in the bile within the first 15 minutes in every instance, 41.9 to 69.8 per cent being excreted during the first hour after a single injection. These values are in accord with previous reports from this laboratory (14, 3). What was said above regarding the promptness of biliary excretion of bile pigment apparently applies equally to biliary excretion of bromsulfalein.

There was no evidence that the excretion of pigment was depressed during the period of maximal excretion of BSP. In fact, the amount of pigment excreted during this period exceeded the control level in every instance but one (table 3, *dog 7*).

TABLE 3. BILIARY EXCRETION OF BSP AND PIGMENT FOLLOWING SINGLE INTRAVENOUS INJECTION OF BSP

		BROMSULFALEIN												PIGMENT EXCRETION			
	Injected	Recovered				Maximum concentration (15-min. periods)				Maximum output (15-min. periods)							
DOG	mg/kg.	total	1st hr.	2nd hr.	3rd hr.	total	mg.%	total	vol.	time	total	mg.%	vol.	time	control	1st hr.	2nd hr.	3rd hr.
		mg.	%	%	%	%		mg.	cc.	min.	mg.		cc.	min.	mg.	mg.	mg.	mg.
4	5	75	52.5	11.3	1.6	65.4	816	17.1	2.1	30	17.1	876	2.1	30	1.373	2.936	2.767	1.526
	5	75	60.4	11.4	2.2	74.0	864	18.1	2.1	45	18.1	864	2.1	45	2.132	2.742	3.062	2.192
	5	75	59.8	11.7	1.9	73.6	790	18.9	2.4	30	18.9	790	2.4	30	1.604	2.178	2.495	2.783
5	5	90	69.8	17.3	9.8	96.9	1280	6.4	0.5	115	24.7	476	5.2	30	1.784	5.118	1.550	3.312
	5	90	55.9	10.1	3.2	69.2	1432	18.6	1.3	60	22.2	1169	1.9	45	4.076	5.037	0.835	1.089
6	5	100	48.2	13.0	2.2	63.5	974	10.7	1.1	60	19.8	903	2.2	30	3.202	3.316	1.810	0.554
	5	100	41.9	15.3	2.8	60.0	750	1.5	0.2	75	11.7	405	2.9	30	3.005	3.768	2.179	3.678
	5	100	42.0	7.4			673	22.8	3.4	45	22.8	673	3.4	45	3.928	4.925	1.250	
	5	100	46.3	7.6	4.3	64.2	1110	11.1	1.0	60	22.9	1044	2.2	45	4.111	4.965	1.573	3.702
7	5	60	49.3	19.8	1.8	70.9	923	12.9	1.4	45	12.9	923	1.4	45	3.475	2.949	3.674	2.725
9	5	70	41.7	11.3	2.6	55.6	292	8.7	3.0	45	11.7	274	4.3	30	3.061	3.587	4.008	4.359

There is, therefore, no indication of competition between BSP and bilirubin for a common excretory mechanism at normal serum bilirubin concentrations.

Simultaneous bilirubin and bromsulfalein injection. After a single priming dose, bilirubin and BSP were injected simultaneously continuously for 90 to 120 minutes. The pertinent data are presented in table 4. In only one case (*dog 10*) was the period of maximum output of pigment and dye delayed as compared with the findings after individual injections of bilirubin and BSP. Approximately the same proportion of dye was recovered (50.1–96.1 per cent) as after prolonged injection of BSP alone (48–74.7 per cent). However, the proportion of injected bilirubin recovered as biliary pigment (40.9–52.5 per cent) was lower than when bilirubin alone was injected (61–100 per cent). This suggests that, at elevated serum bilirubin concentrations,

bilirubin and BSP may compete for a common excretory mechanism, the BSP being excreted preferentially under these circumstances.

Dragstedt and Mills (15) reported that intravenous injection of bilirubin interfered with removal of BSP from the blood. The data presented here (also table 5) indicate that artificially induced hyperbilirubinemia and increased hepatic excretion of bile pigment do not retard the excretion of BSP in the bile. It is known moreover, that the rate of removal of BSP from the blood may be normal in uncomplicated clinical or experimental hemolytic jaundice.

The data presented in table 5 indicate the quantitative discrepancy between the rate of removal of injected bilirubin and BSP from the circulating plasma and the rate of their excretion in the bile. The total quantity in the plasma was estimated on

TABLE 4. BILIARY EXCRETION OF BSP AND PIGMENT DURING AND AFTER PROLONGED INTRAVENOUS INJECTION OF BSP AND BILIRUBIN

DOG	PIGMENT + DYE INJECT.		TEST PERIOD		RECOVERY		MAXIMUM CONCENTRATION (15-MIN. PERIOD)				MAXIMUM OUTPUT (15-min. period)			
	mg./kg	total	injection	post-injection	during injection	total	mg.%	total	vol.	time	total	mg.%	vol.	time
		mg.	min.	min.	%	%	.	mg.	cc.	min.	mg.		cc.	min.
Bromsulfalein														
1	5.5	55.2	120	120	30.1	55.6	642	1.99	0.31	180	4.68	624	0.75	75
2	4.8	48.2	90	120	49.3	73.8	200	5.8	2.9	45				
3	5.2	110	120	120	36.9	50.1	178	7.48	4.2	45	8.33	167	5.0	30
10	5.0	74.6	120	105	70.1	96.1	805	7.84	1.8	120				
Pigment														
1	5.5	55.2	120	120	22.7	41.4	684	2.12	0.31	180	3.95	527	0.75	75
2	4.8	48.2	90	120	38.1	52.5	150	4.35	2.9	45				
3	5.2	110	120	120	26.6	40.9	151	7.53	5.0	30				
10	5.0	74.6	120	105	28.7	41.3	530	1.13	0.2	195	7.84	435	1.8	120

the basis of 50 cc. of plasma per kilogram of body weight. At the end of a two-hour injection period, 43 to 71 per cent of the bilirubin and 34.6 to 63 per cent of the BSP had been removed from the blood stream but had not been excreted in the bile. In no case was a significant amount of either detected in the urine during the period of the experiment. Even if one assumes a distribution of these substances throughout the extracellular fluid at their plasma concentrations, which is highly improbable (7), and also takes into consideration the 'dead space' of the bile duct system, a considerable fraction of the injected pigment and dye remains unaccounted for. If the liver is the only organ involved in their removal from the blood, this implies a subsequent phase of temporary storage in that organ prior to their passage into the bile. It has been suggested (16–19) that the reticuloendothelial system may be involved in the removal of BSP from the blood. There is no clear evidence, however, that extrahepatic tissues are involved significantly in the removal of bilirubin from the blood. The data presented here suggest that these substances are excreted in essentially the

same manner and, when introduced into the circulation, are removed from the body rather promptly, and in large amounts, by the liver. In the light of these findings,

TABLE 5. PERCENTAGES OF BSP AND BILIRUBIN REMOVED FROM THE BLOOD BUT NOT EXCRETED IN THE BILE DURING INTRAVENOUS INJECTION OF BSP AND BILIRUBIN

DOG	WT.	TIME	BILIRUBIN							BROMSULFALEIN						
			Amt. Injected	Excreted		Circulating		Removed, not excreted		Amt. injected	Excreted		Circulating		Removed, not excreted	
	kg.	hr.	mg.	mg.	%	mg.	%	mg.	%	mg.	mg.	%	mg.	%	mg.	%
1	9	½	24.75	3.97	16	2.25	9.0	18.5	75							
		1	31.50	11.64	37	2.25	7.0	17.6	56							
		1½	38.25	18.26	48	2.25	6.0	16.7	46							
		2	45	23.7	53	2.00	4.0	19.3	43							
3	21	½								128	26.5	20.7	4.2	3.3	97.3	76
		1								168	69.2	41.2	4.4	2.8	94.4	56
		1½								208	99.7	47.9	4.2	2.1	104.1	50
		2								253	130.7	51.6	3.7	1.4	118.6	47
4	15	½								43.6	8.0	18.3	2.0	4.7	33.6	77
		1								57.2	24.6	43	2.0	2.5	30.6	53.5
		1½								70.8	36.5	51.5	2.0	2.9	32.3	45.6
		2								84.6	45.5	53.8	2.0	2.3	37.1	43.9
4	15	½								85.5	23	26.9	4.5	5.3	58	67.8
		1								111.0	64.5	58.2	4.0	3.5	42.5	38.3
		1½								136.5	84.2	61.7	3.75	3.1	48	35.2
		2								162.0	103.4	63.8	2.6	1.6	56	34.6
2	10	½								26.25	2.55	9.7	2.0	7.3	21.7	83
		1								32.5	8.8	27.1	2.0	6.9	21.7	66
		1½								38.75	13.15	33.9	2.0	5.1	23.6	61
		2								45.0	26.1	58	2.0	4.0	16.9	38
1	10	½	27.3	0.82	3	3	11.0	23.5	86	27.3	0.33	1.2	2	7.3	25	91.5
		1	36.6	3.5	9.8	3.5	9.2	29.6	81	36.6	4.6	12.6	2	5.4	30	82
		1½	45.9	9.7	21	3	7.0	33.2	72	45.9	13.1	28.5	2	8.5	30.8	63
		2	55.2	12	22	3	7.0	39	71	55.2	16.7	30.2	2	6.8	35	63
3	21	½	59	7.9	13	4.5	8.0	46.5	79	59	8.66	14.6	2.6	4.9	47.5	80.5
		1	76	16.56	22	5	6.0	54.5	72	76	22.23	29.2	2.6	3.8	51	67
		1½	93	23.41	25	3.6	4.0	66	71	93	32.34	34.7	0.1	0.3	60.5	65
		2	110	30	27	4.6	6.5	73	66.5	110	40.6	36.9	2.6	4.1	65	59

TABLE 6. BILIARY EXCRETION OF PIGMENT DURING AND AFTER INTRAVENOUS INJECTION OF LAKED RED BLOOD CELLS

DOG	CONTROL PERIOD			PACKED CELLS INJECTED	INJECTION PERIOD (60 MIN.)			POST-INJECTION PERIOD					
	Pigment excretion				Pigment excretion			First hour			Second hour		
	vol.	pigment			vol.	pigment		vol.	pigment		vol.	pigment	
	cc/hr.	mg. %	mg/hr.	cc.	cc/hr.	mg. %	mg/hr.	cc/hr.	mg. %	mg/hr.	cc/hr.	mg. %	mg/hr.
9	3.4	37.6	1.404	50	4.7	65.5	3.173	8.8	235.7	20.693	10.2	285.6	28.246
	5.3	37.2	1.931	45	7.0	68.6	4.918	10.9	238.0	26.518	11.8	321.6	38.509

the observation of Cohn *et al.* (20) that the peripheral tissues are capable of removing considerable amounts of BSP from the blood in the absence of the liver cannot be

interpreted as indicative of the importance of extrahepatic tissues in this connection in the intact animal.

Hemoglobin injection. Fifty cc. and 45 cc. of packed red blood cells were laked and injected intravenously at an interval of two months. The pertinent data are presented in table 6. The content of bilirubin-precursor (protoporphyrin) in these quantities of laked cells was approximately 600 mg. and 540 mg., respectively. The volume of bile increased steadily during the 3-hour experimental period. A definite increase in pigment excretion occurred in the fourth 15-minute sample in the first experiment and in the third sample in the second experiment. Both the concentration and total quantity of pigment in the bile increased progressively during the experimental period. A similar observation was reported by Greene and Snell (11) in an acute experiment. These data emphasize the rapidity of transformation of intravenously injected hemoglobin to bilirubin and the promptness of excretion of the latter in the bile. In view of the magnitude of the biliary 'dead space' (about 5 cc.) in relation to the 15-minute bile volume (1.4–1.9 cc. during the injection period), it is apparent that these phenomena must be initiated almost immediately after the introduction of hemoglobin into the circulation.

<div align="center">SUMMARY</div>

Determinations were made of the rate of bile flow and pigment excretion in trained, Thomas-type tubulated duodenal-fistula dogs, bile being obtained by inserting a temporary glass cannula into the common duct through the duodenal opening. There was considerable daily variation in the rate of bile flow and of pigment excretion in untreated animals.

After both single and prolonged intravenous injections of bilirubin, there was a prompt increase in the rate of pigment excretion in the bile, accomplished chiefly by an increase in concentration. After a single injection of one mg/kg., 48.9 to 89 per cent of the amount administered was excreted in the bile in three hours, the largest proportion usually in the first hour. Sixty-one to one hundred per cent of the quantity administered by prolonged injection was excreted in four hours.

Bromosulfalein, injected intravenously, appeared promptly in the bile and was recovered in approximately the same proportions as was bilirubin. Pigment excretion was not depressed during the period of maximal excretion of BSP.

When bilirubin and BSP were injected simultaneously, BSP was excreted as efficiently as when administered alone, but the proportion of injected bilirubin recovered as biliary pigment was lower than when bilirubin alone was injected. This suggests that, at elevated serum bilirubin concentrations, bilirubin and BSP may compete for a common excretory mechanism, the BSP being excreted preferentially.

The quantity of bilirubin and BSP removed from the blood stream at any time during prolonged injection greatly exceeded the quantity excreted in the bile up to that time. If the liver is the only organ involved in the removal of these substances from the blood, this implies a phase of temporary storage in that organ prior to their passage into the bile.

Intravenous injection of laked red blood cells resulted in a prompt increase in

pigment excretion in the bile, indicating the rapidity of transformation of hemoglobin to bilirubin under these circumstances.

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RENAL FUNCTION IN NORMAL RABBITS AND DOGS AND THE EFFECT OF URANYL SALTS[1]

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IT HAS been shown by MacNider (1) that the kidney is the organ affected predominantly by administration of uranium to animals. Accordingly, some investigations of the renal action of uranium were carried out as part of an extensive study of the effects of this metal on mammals. This paper is confined to the effects of uranium on the handling by the kidney of chloride, inulin and diodrast—the three substances with which most work was done.

METHODS

Exposures to inhalation of uraniferous dusts were carried out in closed chambers by methods to be described elsewhere (2). In general, the entire animal was exposed to the dusty atmosphere of approximately constant composition for roughly six hours a day on six days of the week. Duration of inhalation exposure is stated in total hours. Animals injected with uranyl acetate received intravenous doses of a 0.3 per cent aqueous solution.

Clearance Determinations—Rabbit. The animal was anesthetized with nembutal (Veterinary Solution), the dose being derived from a curve similar to that of Bazett and Erb (3) but shifted downward so that a rabbit of 3-kg. body weight received 1.8 ml. of solution. Additional anesthetic was given as required to maintain an approximate plateau of anesthesia (injection of 0.2 ml. of solution every 30 min. usually had the desired effect on a 2.5 kg. rabbit).

Cannulas were tied into the trachea, the left saphenous vein and the bladder in that order. As soon as the venous cannula had been inserted, infusion of the animal with a saline solution containing inulin (0.25%) and diodrast (0.52 vol.% of a 30% solution) was started at the rate of 25 ml/kg/30 min. After priming for 30 minutes, the infusion rate was lowered to one ml/min. for the duration of the experiment.

After an equilibration period of at least one hour from the end of priming, collection of successive urine samples at 30-minute intervals was started. Arterial blood samples were taken from one deep femoral artery at the midpoints of the periods of urine collection. At least two determinations were made on each animal.

Table 1 contains sample data to illustrate the rabbit experiments. It shows

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that we were able to maintain fairly constant blood levels of the substances studied and that in the control experiments urine flow was fairly constant for the most part. After injection of uranyl acetate, the urine rate became more variable as a result of the action of the metal on the nephron.

Clearance Determinations—Dog. Females were used, the animal lying on its side on a table. Restraint ties were used only when necessary. The dog was catheterized with a soft-rubber, wing-tip catheter and a hypodermic needle was taped in place in one leg vein.

The dog was primed by intravenous infusion for 30 minutes at the rate of 3 ml/min. with a sterile saline solution containing inulin (0.7%) and diodrast (1.4

TABLE 1. DATA FROM TYPICAL CONTROL AND POISONED RABBITS OBTAINED DURING THE STUDY OF THE ACUTE EFFECTS OF INTRAVENOUS INJECTION OF URANYL ACETATE

TIME	PLASMA CHLORIDE	PLASMA DIODRAST	PLASMA INULIN	URINE CHLORIDE	URINE DIODRAST	URINE INULIN	URINE RATE	CLEARANCE CHLORIDE	CLEARANCE DIODRAST	CLEARANCE INULIN
	mg. %	*mg. 1 %*	*mg. %*	*mg. %*	*mg. 1 %*	*mg.%*	*ml/min.*	*ml/min.*	*ml/min.*	*ml/min.*
				15/12/44 Control (3.75 kg.)						
1:00–1:30	420	1.92	21.0	1007	510	1320	0.29	0.70	77.1	18.4
1:30–2:00	422	1.92	21.1	850	352	880	0.32	0.64	58.7	13.3
2:00–2:30	427	1.93	21.3	773	350	880	0.34	0.62	61.6	14.2
2:30–3:00	433	1.92	21.1	850	447	1120	0.28	0.55	65.2	14.9
3:00–3:30	440	1.86	20.5	898	385	944	0.32	0.65	66.4	14.7
3:30–4:00	444	1.81	20.2	510	169	420	0.66	0.76	61.6	13.8
				12/2/45 (2.56 kg.)						
1:00–1:30	416	6.07	40.2	410	126	264	2.00	1.97	41.5	13.1
1:30	3 mg./kg. UO₂ Ac₂ I.V.									
1:30–2:00	430	6.10	42.2	438	158	320	1.12	1.14	29.0	8.5
2:00–2:30	430	6.00	42.8	481	124	320	1.58	1.77	32.7	11.9
2:30–3:00	431	5.94	43.3	469	120	292	1.67	1.81	33.8	11.2
3:00–3:30	431	5.84	43.8	460	139	332	1.36	1.45	32.4	10.3
3:30–4:00	428	5.85	43.9	491	124	325	1.61	1.85	34.2	11.9
4:00–4:30	426	5.90	44.1	467	136	328	1.42	1.56	32.8	10.6
4:30–5:00	423	5.83	44.2	467	128	332	1.43	1.58	31.4	10.7

vol.% of a 35% solution). At the end of the priming period, infusion was continued at the same rate but with a solution containing half the above concentrations of inulin and diodrast. Equilibration was allowed for at least an hour after the priming period before starting collection of clearance samples. Four consecutive urine samples were collected at 15-minute intervals, the bladder being washed out with 10 ml. of warm, sterile-distilled water at the end of each period. A venous blood sample was taken at the midpoint of each period.

The dog experiments were carried out under our direction by Drs. J. Roberts, D. C. Brodie, D. L. Adler and Mr. J. Tournaben. The chemical analyses of the samples and the analysis of the results were made by ourselves.

ANALYTICAL METHODS

Urine and oxalated plasma samples were analyzed for chloride by methods of Peters and Van Slyke (4, pp. 833–834). Their inulin concentrations were estimated by the method of Harrison (5). Fermentable reducing substances in both urine and plasma were separated from the non-fermentable by the method of Van Slyke and Hawkins (6).

Urine and whole blood were analyzed for diodrast by the method of Alpert (7). According to Bobey and Price (8), appreciable amounts of diodrast are held by the precipitated red cells when whole dog blood is analyzed by this method. This observation has been checked by us and extended to the rabbit. In our work it was found that the plasma diodrast level could be calculated from the whole blood analysis for the dog by equation 1 and for the rabbit by equation 2.

$$P_D = \frac{100 W_D}{90.6 - 46.7 V_c + 5.8 V_c^2} \qquad 1$$

$$P_D = \frac{100 W_D}{96.6 - 93.4 V_c + 6.6 V_c^2} \qquad 2$$

Here P_D is the plasma concentration of diodrast in mg. per cent, W_D is the whole blood concentration of diodrast and V_c is the cell fraction determined by hematocrit.

Interpretation of Data. The analytical data and the figures for rate of urine flow were used for calculation of urinary clearances in ml/min. As a measure of tubular function we have used the ratio of the amount of substance transferred across the tubular membrane to that transferred across the glomerular one. For reabsorbed substances, like chloride, this function is calculated by equation 3.

$$f_{Cl} = 1 - \frac{U_{Cl} P_{In}}{U_{In} P_{Cl}} \qquad 3$$

while for secreted materials, like diodrast, equation 4 is used

$$f_D = \frac{U_D P_{In}}{U_{In} P_D} - 1 \qquad 4$$

Here U is the urinary concentration, P is the plasma concentration and the subscripts Cl, D and In refer respectively to chloride, diodrast and inulin.

Chloride has been found to be reabsorbed partially in the proximal convolution and partly in more distal portions of the nephron (9). Since filtered chloride is reabsorbed almost completely, a decrease in the f_{Cl} would be a fairly sensitive indicator of interference with tubular function but would not tell anything about the locus of the action.

On the other hand, a decrease in the f_D indicates not only interference with tubular function but, also, to some extent the locus of the action. Since phlorizin lowers the ability of the tubule to transport diodrast (10) and since the tubular transports of glucose and diodrast are affected similarly by thyroxin (11), there is a good probability that diodrast is secreted at least in part in the same portion of the nephron as is concerned with reabsorption of glucose. Glucose is stated to be reabsorbed almost completely in the first half of the proximal convolution (9). Thus, a decrease in f_D is believed to indicate impaired function in the proximal segment.

The possibility of change in the physical properties of the nephron, within a few days after poisoning with uranyl salts (12), makes it advisable to use only immediate effects on renal function in attempting to identify the locus of uranium action on the kidney. The same factor makes values of the filtration fraction of doubtful significance some days after the injection of uranium salt.

RESULTS

Renal Function in Normal Rabbits and Dogs. In normal animals, all the measures of renal function studied here appeared to be correlated to some extent with the rate

TABLE 2. EQUATIONS OF LEAST SQUARE LINES EXPRESSING THE COVARIANCES OF URINE RATE AND THE VALUES OF VARIOUS RENAL FUNCTIONS IN CONTROL ANIMALS

FUNCTION	RABBIT		DOG	
	Equation	No. of Points	Equation	No. of Points
C_{Cl}	$0.04 + 1.08u$	48	$0.73u - 0.31$	61
C_D	$50.6 + 13.7u$	29	$212 - 0.67u$	62
C_{In}	$12.6 + 0.91u$	45	$53.6 + 3.0u$	54
f_{Cl}	$1.00 - 0.07u$	23	$1.00 - 0.01u$	51
f_D	$2.02 + 0.66u$	28	$2.96 - 0.16u$	52

Clearances (C) in ml/min. u = urine rate in ml/min. f = tubular transport per unit of filtration, defined by text equations 3 and 4.

TABLE 3. EXAMINATION OF THE SIGNIFICANCE OF THE LEAST SQUARE LINES OF TABLE 2.

FUNCTION	RABBIT						DOG					
	S_y	$u_{min.}$	X_1	$u_{max.}$	X_2	$\dfrac{X_2 - X_1}{S_y}$	S_y	$u_{min.}$	X_1	$u_{max.}$	X_2	$\dfrac{X_2 - X_1}{S_y}$
C_{Cl}	0.22	0.04	0.08	2.42	2.66	11.7	0.47	1.3	0.68	9.5	6.58	12.5
C_D	20.3	0.05	51.0	2.42	84.0	1.6	50	1.3	211	9.5	205	0.1
C_{In}	4.8	0.02	12.6	2.42	14.9	0.5	8.9	1.3	57.5	9.5	81.8	2.7
f_{Cl}	0.04	0.04	0.99	2.42	0.83	4.0	0.01	1.3	0.99	9.5	0.92	7.0
f_D	1.05	0.16	3.13	2.42	4.60	1.4	0.64	1.3	2.75	9.5	1.48	2.0

S_y = standard error of estimate. u_{min} and u_{max} = minimal and maximal experimental rates of urine flow, in ml/min. X_1 and X_2 = values on regression line corresponding respectively to u_{min} and u_{max}.

of urine flow. Table 2 gives the equations of the least square lines for the relations between the various parameters and the urinary rate in our experiments. The lines of table 2 were examined for significance by the following steps: *a*) calculation of the standard error of estimate (S_y), *b*) calculation of the difference between the regression values at the extreme experimental urine flows and *c*) calculation of the ratio of the value found in *b* to that found in *a*. The results of this process are given in table 3.

It is seen from table 3 that in our experiments the only variables having clearly significant correlations with urine flow are the clearance of chloride and the reabsorption of chloride. This holds for both the dog and the rabbit, although in the dog there appears to be a fair chance (143:1) that the inulin clearance also is a function

of the rate of urine flow. The latter finding is of interest in that it shows a clear difference between our series of experiments and that of Shannon (13) in which the latter author found no relationship between glomerular filtration in the dog and the rate of urine flow. Our data on inulin clearance in the rabbit differ from those of Kaplan and Smith (14) in that we found no significant relationship between the clearance and the urine rate while Kaplan and Smith thought that there was such a correlation. The reason for our unorthodox results with inulin clearance is not apparent.

Although the relation of most of our parameters to urine rate was not of high order, it appeared that it would be more correct to compare our experimental values to those obtained from these regression lines than to compare them to a mean normal value. Accordingly, in assessing the effects of administration of uranium compounds upon renal function, the appropriate lines of table 2 were used to evaluate the expected levels of the variables at the experimental rates of urine flow. The experimentally determined values of the parameters were then compared with the expected ones.

Acute Effects of Intravenous Injection of Uranyl Acetate in the Rabbit. The typical response of urine flow to intravenous injection of uranyl acetate was an immediate increase followed by a slow decrease. The urine rate fell from its peak more rapidly with high doses than with small ones. The upper curve of figure 1a, in which the urine rate at 2 hours 10 minutes after the injection (expressed in percentage of the control rate) is plotted against the dose of uranyl acetate, shows that the maximal increase in urine flow is produced by a dose of about 0.5 mg/kg. of the uranium salt.

For comparison with our curve there is drawn on this figure a graph derived from data of Watanabe, Oliver and Addis (15) showing the effects of various doses of uranyl acetate upon the secretion of urine 72 hours after administration of the salt. These two graphs show that the dose of uranyl acetate which produced the maximal acceleration of urine flow in our short-time experiments also produced some acceleration of urine rate 72 hours after the injection. Doses of the uranyl salt greater than 1 mg/kg. produced some increase in urine flow 2.17 hours after the injection, but produced oliguria or anuria within three days.

The acute effects (2.17 hours after the injection) of intravenous injections of uranyl acetate upon renal clearances are shown in figures 1b, 1c and 1d. The only clearly significant change shown here was a decrease in the diodrast clearance.

The decreased clearance of diodrast could be the result of decreased blood flow through the kidney but this is believed not to be the case. Unpublished work of D. L. Adler in this laboratory has shown that intravenous injection of uranyl acetate does not decrease blood flow through the frog glomerulus. Direct measurements of blood flow through the mammalian kidney (12, 16) have shown also that injection of uranyl salts does not decrease the circulation through the kidney. This leaves as the alternative explanation for the decrease in diodrast clearance after injection of uranyl acetate some interference with the secretory ability of the tubular epithelium.

Figure 1e shows that there was no immediate diminution in chloride reabsorption although there was in diodrast secretion. These findings suggest that the uranyl salt interferred with the function of the proximal convolution of the nephron.

Subacute Effects of Inhalation of Uranyl Fluoride by the Rabbit. Figure 2 shows

the results of renal function studies on rabbits exposed to atmospheres of various concentrations of uranyl fluoride dust for different periods of time. Each point represents a different animal, so that the large peaks in the uppermost curves of figures 2b and 2c probably have no significance.

Figure 2 demonstrates that the clearance of chloride was not affected by any of the dust concentrations used although the clearances of diodrast and inulin were reduced definitely by the largest one. The latter two clearances may have been reduced by the intermediate concentration of dust. The lowered clearance of inulin
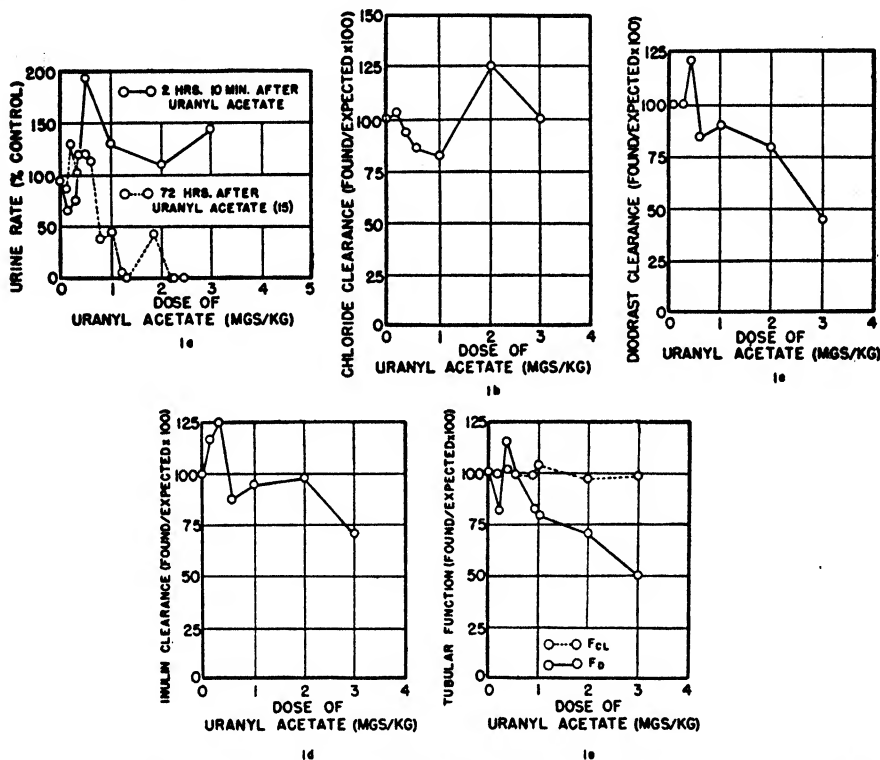
Fig. 1. IMMEDIATE (2 hrs., 10 min.) EFFECTS OF INTRAVENOUS DOSES of uranyl acetate upon the rate of urinary excretion and upon renal function. Each point represents an average of one determination on each of 3 rabbits.

during the dust exposure, particularly marked at the 2.78 mg/M³ level, does not mean necessarily that there was a decreased rate of filtration through the glomerulus (12). It may mean, rather, that the tubular epithelium had been so altered by the action of the uranium as to be permeable to substances which ordinarily are unable to pass from the tubular lumen into the peritubular capillaries.

When tubular function in these three inhalation experiments was assessed by *equations 3* and *4*, the curves of figures 2d and 2e were obtained. These graphs show that chloride reabsorption was unaffected except by the highest dust concentration although there appears to have been some effect on diodrast secretion by all dust concentrations. The decrease in diodrast secretion appears to have been lesser in magnitude and later in appearance with the smaller concentrations of uranyl fluoride dust.

It is evident from figure 2 that renal function goes through a minimum during continued exposure to atmospheres containing uranyl fluoride. In nonfatal exposures the maximal effect is produced early in the exposure and is followed by partial recovery of renal function. The extents of effect and recovery and the time courses of these actions vary with the function studied. Thus, it is evident from figures 2d
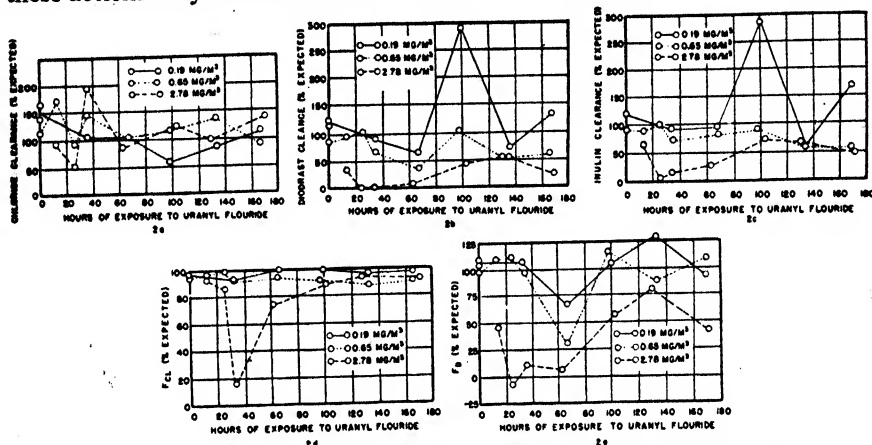
Fig. 2. EFFECTS UPON RENAL FUNCTION of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time. Each point represents the mean of two successive determinations upon a single rabbit.
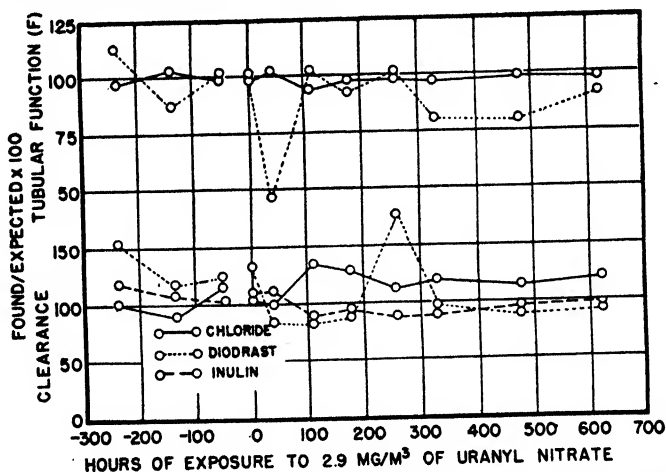
Fig. 3. EFFECT UPON RENAL FUNCTION of *dog 695* of exposures to an atmosphere containing 2.9 mg/M³ of a dust of uranyl nitrate. Each point represents the mean of 4 successive determinations.

and 2e that the secretion of diodrast was affected more markedly than the reabsorption of chloride and that the maximal effect on diodrast secretion occurred earlier and persisted longer than that on chloride reabsorption. This temporal difference between the minima in chloride reabsorption and diodrast secretion may mean that these two processes occur by different mechanisms in the tubule.

Subacute Effects of Inhalation of Uranyl Nitrate by the Dog. Three dogs were

exposed to an atmosphere containing an average of 0.3 mg/M³ of uranyl nitrate dust and 2 to one containing an average of 2.9 mg/M³. The animals were placed in the dusty atmospheres for about six hours a day, the rest of their time being spent in normal room air.

Figure 3 shows the results on one of the dogs exposed to the high level of dust. It is obvious that in the dog exposed to uranyl nitrate the effects of the exposure are the same qualitatively as in the rabbit exposed to uranyl fluoride. Again it is apparent that the secretion of diodrast is affected more than the reabsorption of chloride and that the clearance of diodrast is the only clearance reduced appreciably by the exposure.

Because the secretion of diodrast appears to be the most sensitive of the measures used here, table 4 summarizes the effects of the two levels of exposure to uranyl nitrate upon f_D of the dog. It can be seen that the low level of exposure probably had no significant effect on the ability of the tubules to transport diodrast. The high level of exposure, however, had a definite effect in decreasing the ability of the tubule to secrete diodrast.

TABLE 4. EFFECT OF INHALATION OF URANYL NITRATE DUST UPON THE SECRETION OF DIODRAST BY THE TUBULE OF THE DOG

DUST LEVEL	DOG NO.	f_D (FOUND/EXPECTED $\times$ 100)		HRS. OF EXPOSURE FOR MINIMAL f_D
		Av. Pre-Exposure	Min. during Exposure	
0.3 mg./M³	575	95	70.8	36 and 468
0.3 mg./M³	636	99	83.2	258
0.3 mg./M³	666	101	81.0	468
2.9 mg./M³	656	71	−25.3	48
2.9 mg./M³	695	134	47.9	36

DISCUSSION

Our findings about renal function in the normal dog and rabbit agree in general with those already in the literature. Our results differ from the literature in the previously discussed finding that inulin clearance in the dog might be correlated with urine rate while in the rabbit it was not. This reversal of the classical situation may depend on our experimental conditions but we are unable to identify the responsible factor.

All of the experiments reported here indicate that the effect of uranyl salts on renal function is the result of interference with the structural and functional integrity of the proximal convolution of the tubules. The fact that reabsorption of chloride is affected only moderately as compared with the secretion of diodrast suggests that the action of uranium is restricted to the proximal convolution rather than affecting both convolutions of the nephron. The microdissection studies of Oliver (17) have localized the histological uranium action in the latter part of the proximal segment.

The negative values of f_D obtained in some rabbits (fig. 2e) and dogs (*dog 656*, table 4) indicate that not only has the ability of the tubule to secrete diodrast been removed completely, but also the normal barrier to diffusion of filtered diodrast from the tubular lumen has been broached in part. Comparison of figures 2c and 2e

shows that the only rabbits showing a decreased inulin clearance were the ones with markedly decreased ability to secrete diodrast. This correlation indicates that uranium brought about an increased permeability of the tubular epithelium since it has been pointed out already that probably the blood flow to the kidney, and consequently the filtration, does not change.

CONCLUSIONS

1. In the kidney of the normal dog and rabbit the clearance of chloride and the reabsorption of chloride by the tubule are functions of the urine rate; the value of the chloride clearance increases with urine rate and the fractional reabsorption of chloride decreases slightly. The ability of the kidney of the normal dog and rabbit to clear diodrast from the blood by secretion is not a function of urine rate. Under the conditions of our experiments the inulin clearance appears to be a function of urine rate in the dog but not in the rabbit. Uranyl salts affect the function of the proximal convolution, this effect leading to decreased reabsorption of chloride, decreased secretion of diodrast and probably to increased permeability of the tubular membrane to water and dissolved substances.

ADDENDUM

Since the completion of this work in June 1946, Lotspeich, Swan and Pitts (18) have studied the renal tubular reabsorption of chloride. They found that in the dog the rate of tubular reabsorption of chloride is a direct, linear function of the rate of glomerular filtration. Their line had a slope of nearly one, agreeing thereby with our findings that the fractional reabsorption of chloride decreases only slightly with increasing urine flow. This slight decrease is significant, however, for both dog and rabbit.

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UNILATERAL ADRENALECTOMY, UNILATERAL SPLANCH-
NIC NERVE RESECTION AND HOMOLATERAL
RENAL FUNCTION[1]

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IN 1914 Cow (1) reported the anatomical and physiological demonstration of a vascular connection between the adrenal gland and the homolateral renal capsule of the cat. It was postulated that via this communication adrenalin could reach the kidney without first passing through the general circulation. Removal of one adrenal gland resulted in markedly increased production of urine by the kidney on the side operated upon compared with the urine flow on the unoperated side. The blood flow in the kidney homolateral to the excised adrenal was not found increased in proportion to the flow of urine. Cow explained the effects observed as due to the failure of adrenalin to reach the homolateral kidney via the direct adrenal-renal circulation. Marshall and Kolls (2) subsequently observed in dogs an increased excretion of water, chloride and urea by the kidney on the side on which adrenalectomy had been performed, but the simultaneous homolateral excretion of creatinine, phenolsulphonephthalein and lactose was approximately the same or only slightly increased as compared to the normal side. Because similar results were obtained by unilateral section of the splanchnic nerve and by section of the renal nerves, Marshall and Kolls concluded that unilateral removal of the adrenal gland affects the kidney on the same side only in so far as nerves associated with the kidney are injured.

Most evidence (3) favors the concept that the renal nerves have no direct influence on renal tubular reabsorptive or excretory functions. Therefore, it may be presumed that if, as suggested by Marshall and Kolls, injury to the renal nerves is responsible for the effects of adrenalectomy upon excretion of water and solids described above, these effects occur in association with the increased rate of *renal blood flow* which denervation of the kidney occasions in the anesthetized animal (4-6). However, the methods available to Marshall and Kolls afforded little evidence of a consistently increased rate of blood flow or glomerular filtration as a result of unilateral adrenalectomy. The creatinine clearance has been shown to be a valid measure of glomerular filtration rate in the dog (6, 7) and the phenolsulphonephthalein clearance is roughly correlated with the renal blood flow (6, 8). Thus, the failure of a markedly increased excretion of creatinine or phenolsulphonephthalein to accompany consistently the unilateral diuresis of water and chloride

observed in the unilaterally adrenalectomized dog (2) suggests that the diuresis observed by Marshall and Kolls was not dependent on differences in *glomerular filtration rate or renal blood flow*.   Cow's anatomical and physiological findings, and the subsequent demonstration (9, 10) of the marked influence of the adrenal glands upon the excretion of sodium chloride and water by the kidneys, seem to justify reexamination of Marshall's and Koll's contention that the adrenal gland does not influence specifically the function of the homolateral kidney.   Renal clearance techniques afford the opportunity to investigate more thoroughly the effect of unilateral adrenalectomy upon the hemodynamics of each kidney simultaneously and to correlate observed changes with the urinary excretion of water and chloride.

METHODS

Mongrel dogs maintained on a diet of Friskies Dog Food Cubes (Purina) and occasional feedings of horse meat were used in all experiments.

Operative procedures.   Intravenous nembutal (.029 gm/kg. body weight) was the anesthetic agent employed during all operations and clearance determinations.   Using sterile operative technique, the left adrenal gland was exposed retroperitoneally.   The large lumbar vein, which regularly crosses the anterior surface of the adrenal gland to be joined medially by the main adrenal vein, was ligated on both sides of the gland and the adrenal was dissected away from adjacent sympathetic nerves as carefully as possible.   Injury to these nerves during dissection seemed inevitable.

Supradiaphragmatic splanchnic section or sympathectomy were performed while intermittent positive pressure was administered by intratracheal catheter.   In performing the supradiaphragmatic neurectomies, 2 to 5 cm. of splanchnic nerve ('splanchnic resection') and usually of sympathetic chain ('sympathectomy') were removed just above the site of passage of these structures through the diaphragm.   The splanchnic nerve almost always presented itself as a single cord; greater and lesser subdivisions were usually not identified.

Except in the 'acute' experiments, renal clearances were usually measured at least two weeks after the last adrenal or splanchnic operation.   Each ureter was approached retroperitoneally, severed, and a no. 6 French ureteral catheter securely tied in.   The position of the catheter was adjusted carefully throughout the remainder of the experiment to prevent obstruction of urine flow due to angulation of the ureter.

Technique of renal clearance measurement.   Renal clearances were measured with the animal anesthetized with nembutal, in the supine position.   The infusion mixtures usually consisted of *a)* a 'priming' solution of 15 cc. of 25 per cent mannitol solution and 0.5 cc. of 20 per cent sodium para-aminohippurate ('PAH') solution and *b)* a 'sustaining' solution of 1.6 per cent mannitol and 0.12 per cent PAH in 0.9 per cent aqueous solution of NaCl.   The latter solution was administered by continuous intravenous drip at a rate of 3 to 4 cc. per minute.   Urine was collected directly from the ureteral catheters into small volumetric flasks.   Clearance periods were usually 30 minutes long.   An infusion of 100 to 300 cc. of 0.9 per cent saline solution was always given before starting the clearances in order to establish a good urine flow.

In some experiments it was thought desirable to increase the urine flow or the urinary chloride concentration in the later clearance periods.   For these purposes, 100 cc. of 2 per cent or 5 per cent NaCl was administered in place of the usual sustaining mixture at a rate about double that employed for the sustaining mixture, and the concentrations of mannitol and PAH in the hypertonic solutions were accordingly reduced.   Arterial blood samples were collected either from the femoral artery or from the heart at approximately the mid-points of the clearance periods.   The aspirating syringe and the test tube receiving the blood each contained two drops of one per cent heparin solution.   In later experiments (*dog 14* and after) the blood was collected under oil and iced until centrifugation.   Centrifugation of the blood and separation of the plasma were performed usually within an hour after bleeding.

In those clearance experiments in which epinephrine was administered, the animal received 10

to 14 gamma of the hydrochloride per minute. Where pituitary adrenocorticotropic hormone[3] was administered, an aqueous solution containing 20 mg. was given intravenously over the course of one minute.

Post-mortem examination was performed in all animals in order to verify the presence or absence of adrenal tissue, the interruption of nerve tracts and the presence of grossly normal kidneys bilaterally.

The calculations of the renal clearances of mannitol and PAH were determined according to the method of Möller, McIntosh and Van Slyke (11), except that plasma rather than whole blood was used. Therefore, the term 'clearance' refers to plasma clearance.

Analytical techniques. Mannitol was measured by the periodate-thiosulfate titration technique of Smith *et al.* (12). Cadmium sulfate was used to precipitate protein from plasma and urine, as recommended by Goldring and Chasis (13). Among factors which were considered in performing the plasma and urine blanks were nonfermentable reducing substances contributed by the plasma and urine, or 'Factor 1', reducing substances contributed by the yeast suspension,[4] or 'Factor 2', and adsorption or destruction of mannitol by the yeast suspension, or 'Factor 3'. In performing the plasma blank during determinations of the mannitol clearance for *dogs 1–7* Factors 1 and 2 were compensated for. For *dogs 8–23* a blank was employed which compensated for only Factor 1. All three factors were taken into consideration for *dogs 24–28*. As regards the urine mannitol blank, no blank was applied until the experiment on *dog 22* at which time there was introduced a blank compensating for Factors 2 and 3. A urine blank compensating for all three factors was employed only for *dog 26*. Details of the method employed for calculation of the blank will be presented in a separate report (14).

These variations in technique influenced the calculated values for the renal clearances to a significant degree. However, the effects of variation in the analytical methods, applying equally as they do to the observations made on both kidneys, are not likely to invalidate conclusions based on comparisons of the simultaneous functions of the right and left kidney.

Para-aminohippurate was measured as recommended by Smith *et al.* (15). The plasma blank was determined after addition of a known quantity of PAH to a protein-free filtrate. The urine PAH blank was found to be insignificant in one dog and was not corrected for in any experiment.

The chloride content of plasma was determined as described by Van Slyke (16); that of urine was measured by the Volhard-Arnold technique (17).

RESULTS

In general, in this paper reference to an 'increase' in excretion of any urinary constituent refers to a greater output of that constituent on the side in question *as compared to the contralateral side.* Thus, the term 'increase' does not necessarily refer to an absolute increase. Data on a representative experiment from each of the groups described below are presented in table 1. Average values of excretory rate ratios for all the satisfactory experiments in the various groups are shown in table 2; data obtained following the injection of epinephrine or adrenocorticotropic hormone are excluded from this table.

Controls. The results of studies in 6 control dogs are shown in table 2, the only operation performed being the insertion of the ureteral catheters. In these animals the ratio of the excretion rate of each of the substances for the left kidney to the rate for the right kidney usually approximated unity. Data on an additional control dog were excluded because of the presence of ureteral obstruction, since this factor

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[3] Donated by Armour & Co., Chicago, Ill. This preparation, lot 37-KE, contained 0.0025 U.S.P. units of the oxytoxic factor and 0.005 units of the pressor factor of the posterior pituitary gland per milligram.

[4] Starch-free baker's yeast donated by Anheuser-Busch, Inc., St. Louis, Mo.

TABLE 1. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON SIMULTANEOUS EXCRETORY RATES FOR EACH KIDNEY—REPRESENTATIVE EXPERIMENTS

ANIMAL	OPERATION	PER.	URINE Volume Rt.	Volume Lt.	Chloride Rt.	Chloride Lt.	PLASMA CHLORIDE	PLASMA CLEARANCE Mannitol Rt.	Mannitol Lt.	PAH Rt.	PAH Lt.	EXCRETORY RATE, LT. KIDNEY / RT. KIDNEY Mannitol	PAH	Water	Chloride	COMMENT
			cc. per min.	cc. per min.	mEq. per l.	mEq. per l.	mEq. per l.	cc. per min.	cc. per min.	cc. per min.	cc. per min.					
Dog 6. 31 lbs.	Control	1	0.71	0.69	78.1	71.5	114.7	17.1	16.5	58.6	55.3	0.96	0.94	0.97	0.90	
		2	1.41	1.30	142	138	125.9	17.1	16.8	46.9	43.4	0.95	0.93	0.92	0.90	5% NaCl
		3	1.54	1.45	152	152	124.5	16.5	16.1	45.3	44.6	0.97	0.98	0.94	0.94	
Dog 3. 22 lbs. 17 days[1]	Lt. adrenalectomy, chronic	1	1.36	1.89	68.1	82.9	111.7	25.8	26.6	50.7	55.4	1.03	1.10	1.40	1.70	
		2	0.97	1.88	76.2	110	112.1	29.2	31.4	72.7	79.4	1.08	1.09	1.95	2.79	5% NaCl
		3	1.29	2.20	128	148	122.2	31.5	32.3	92.0	102	1.02	1.11	1.70	1.98	
		4	1.69	2.60	174	178	129.8	29.1	31.4	78.6	94.2	1.08	1.20	1.54	1.57	
		5	1.75	2.19	185	186	129.3	31.0	32.0	80.5	92.0	1.03	1.14	1.25	1.26	
		6	1.65	2.08	196	198						1.05	1.19	1.27	1.27	
Dog 15. 34 lbs.	Lt. adrenalectomy, acute	1	0.69	0.72	146	138	112.5	28.8	29.1	106	108	1.01	1.01	1.04	0.94	
		2	0.58	0.54	137	122	113.1	26.1	25.2	89.7	92.5	0.97	1.03	0.93	0.83	Lt. adrenalectomy; 64 min. elapsed between per 2 & 3
		3	0.51	0.87	54.2	85.5	117.7	27.9	29.4	79.5	81.9	1.05	1.03	1.72	2.71	
		4	0.52	0.97	76.3	104	121.0	24.7	29.2	67.6	75.6	1.18	1.12	1.87	2.55	2% NaCl
		5	0.59	1.08	110	131	123.4	26.8	28.7	73.9	76.4	1.07	1.03	1.83	2.21	5% NaCl
		6	1.35	2.03	156	160	130.7	29.5	31.9	102	109	1.08	1.07	1.50	1.54	
Dog 8. 31 lbs. 13 days[1]	Lt. splanchnic resection; lt. sympathectomy, chronic	1	0.59	2.11	78.3	160	112.7	28.8	34.4			1.19		3.59	7.36	
		2	0.37	1.77	54.0	166	111.9	29.5	32.7			1.11		4.79	14.7	2% NaCl
		3	0.65	1.60	117	169	113.2	31.7	36.0			1.14		2.46	3.55	
		4	0.56	1.33	88.0	153	113.8	33.8	38.5			1.14		2.36	4.11	
		5	0.62	1.03	126	159	114.9	28.1	30.6			1.09		1.66	2.09	Epinephrine
Dog 13. 20 lbs.	Lt. splanchnic resection, acute	1	0.66	0.68	64.6	78.8	113.3	23.1	23.6	66.7	70.9	1.02	1.06	1.02	1.25	
		2	0.72	0.79	93.1	105	114.5	23.3	24.5	66.3	69.7	1.05	1.06	1.09	1.22	Lt. splanchnic resection; 46 min. elapsed between per. 2 & 3
		3	0.51	1.73	66.0	153	116.5	21.2	25.4	62.1	69.7	1.20	1.12	3.38	7.84	
		4	0.98	2.94	120	158	124.4	21.2	22.8	55.8	66.3	1.08	1.19	2.99	3.94	2% NaCl
		5	1.68	4.77	155	176	140.2	20.9	25.0	52.3	61.2	1.20	1.17	2.84	3.23	5% NaCl
		6	1.29	4.45	150	178	137.7	20.1	23.0	49.6	57.5	1.16	1.16	3.46	4.08	
Dog 26. 31 lbs. 28 days[1]	Bilateral splanchnic resection, bilateral sympathectomy, lt. adrenalectomy	1	1.91	1.74	143	142	111.0	33.5	32.3	107	101	0.97	0.95	0.91	0.90	
		2	2.18	1.80	150	146	109.6	33.9	32.8	103	102.	0.97	0.99	0.83	0.81	
		3	2.04	1.73	153	150	107.6	33.9	32.9	102	101	0.97	0.99	0.85	0.83	
		4	2.00	1.49	158	152	109.6	36.3	34.2	105	103	0.95	0.98	0.75	0.72	ACTH[2] 20 mg. I.V. start of per. 4
		5	1.97	1.81	157	165	110.5	33.8	33.5	96.4	96.2	1.00	1.00	0.92	0.97	
		6	1.61	1.40	162	160	110.9	34.5	34.1	104	102	0.99	0.98	0.87	0.86	
		7	1.58	1.35	175	172	111.1	36.8	36.8	115	117	1.00	1.02	0.86	0.85	

[1] Time interval between operation and measurement of excretory rates.   [2] Pituitary adrenocorticotropic hormone.

causes a diminished output of urine and chloride (19, 20) as well as a decreased rate of glomerular filtration (21).

Unilateral adrenalectomy—chronic. In 3 out of 4 dogs, removal of the adrenal gland resulted in a markedly greater excretion of water and chloride and a slightly but consistently greater excretion of mannitol and PAH by the homolateral kidney as compared with the control kidney (e.g., *dog 3*, table 1). In the mannitol excretion ratios of 2 animals there were intermittently observed deviations from unity which were not in excess of the deviations observed in the control animals (compare *dogs 3* and *6*, table 1); however, these variations in the adrenalectomized animals were accompanied by deviations in the excretion of water and chloride which were more striking than those in the control group. In the one animal (*dog 22*) responding atypically, no appreciable increase was demonstrated in any of the urinary constitu-

TABLE 2. INFLUENCE OF VARIOUS OPERATIVE PROCEDURES UPON AVERAGES OF EXCRETORY RATE RATIOS FOR VARIOUS SUBSTANCES

OPERATION	NO. ANIMALS	NO. PERIODS OF OB- SERV.	EXCRETORY RATE, LT. KIDNEY / EXCRETORY RATE, RT. KIDNEY			
			Mannitol	PAH	Water	Chloride
Control.........................	6	14	1.00	1.00	1.02	1.06
Lt. adrenalectomy....................	5	22	1.06	1.08	1.67	2.20[1]
Lt. splanchnic resection[2].............	3	14	1.18	1.19	3.05	5.47
Bilateral splanchnic resection; left ad- renalectomy[3].....................	4	11	1.01[1]	1.01[1]	0.97[1]	0.99[1]

[1] Statistical analysis by a method applicable to small samples (18) indicates that the differences between these values and the control values are not statistically significant ('t' less than 2.5).

[2] The left sympathetic chain was also sectioned in 2 animals in this group.

[3] The sympathetic chain was also sectioned bilaterally in 3 animals in this group.

ents; in the discussion below the explanation will be suggested that a minimum of damage was caused to the sympathetic nerves of this animal during adrenalectomy. Data on 3 other animals in this group were excluded from table 2 because of the presence of ureteral obstruction, severe emaciation, and chronic infection, respectively.

Unilateral adrenalectomy—acute. The effects of unilateral adrenalectomy were evaluated in one animal by comparing the clearances on the two sides immediately before and after excision of the gland (*dog 15*, table 1). The increased excretion of water and chloride on the homolateral side immediately following the operation was striking. As in the case of *dog 3* (table 1) and *dog 4* (unilateral adrenalectomy), these changes were accompanied by only a relatively small increase in mannitol clearance. A summary of data on the 3 animals which had undergone unilateral adrenalectomy is presented in table 2.

Unilateral splanchnic nerve resection—chronic. Since it was known that resection of the splanchnic nerves resembles adrenalectomy in that it also results in an increased output of chloride and water by the homolateral kidney in the anesthetized animal (2, 6, 22), it was desirable to compare the effects of supradiaphragmatic

splanchnic resection with those of adrenalectomy, especially with regard to the clearance of mannitol and PAH.   In 2 dogs resection of the splanchnic nerve and the sympathetic chain resulted in a greater diuresis of chloride and water than was produced by adrenalectomy (e.g. *dog 8*, table 1).

Unilateral splanchnic nerve resection—acute.   In a single acute experiment (*dog 13*, table 1) in which preliminary control clearances were performed, the markedly increased output of chloride and water immediately after splanchnic resection was striking; a moderate increase in PAH and mannitol excretion was also observed. These findings indicate that the acute and chronic effects of unilateral splanchnic resection are similar.   Maintenance of the integrity of the sympathetic trunk in the acute experiment did not qualitatively modify the response noted in the chronic experiment (*dog 8*, table 1).   The data on the 5 animals which underwent unilateral splanchnic resection are summarized in table 2.

Bilateral splanchnic resection and unilateral adrenalectomy.   It soon became apparent that the results following unilateral adrenalectomy were similar to those following unilateral splanchnic resection.   Three explanations for the effect of adrenalectomy therefore seemed possible: 1) unavoidable injury to nerves, 2) interruption of flow of adrenal hormones to the kidney or 3) a combination of these two mechanisms.   In order to distinguish a primary adrenal influence from a nervous influence, a comparable nerve lesion was produced on both sides by a bilateral supradiaphragmatic splanchnicectomy; in addition, unilateral left adrenalectomy was performed.   After bilateral transection of the splanchnic nerves, any effect observed following unilateral adrenalectomy would probably be due to removal of adrenal hormones from the blood supply to the homolateral kidney.   The results of experiments on 4 animals made it apparent that adrenalectomy did not cause a homolateral increase in excretion of chloride and water after splanchnicectomy (table 2, and *dog 26*, table 1).   No consistent correlation between the mannitol excretion ratios and the urine or chloride excretion ratios was observed.

However, there still remained the possibility that the remaining intact, but denervated right adrenal gland in these animals was incapable of discharging epinephrine (25), a substance known to augment the output of adrenal corticosteroids (24, 25) by stimulating the anterior pituitary to produce adrenocorticotropic hormone (26).   Hence, this denervated adrenal may not have produced amounts of cortical hormone sufficient to influence homolateral renal function via the hypothetical direct adrenal-renal vascular channel.   In order to ensure adequate stimulation of the remaining adrenal, pituitary adrenocorticotropic hormone was administered intravenously to 2 of the 4 animals already referred to as having undergone bilateral sympathectomy and unilateral adrenalectomy.   The results in these 2 animals (e.g. *dog 26*, table 1) indicated that adrenocorticotropic hormone exerted no immediate differential effect upon the function of the two kidneys.   Evidence of one aspect of the hormone's biological activity was provided in both animals by the observed increase in the ratio of the excretion of uric acid to that of creatinine, as described by Forsham *et al.* (27).   Epinephrine was administered to the 2 other dogs, in part for the purpose of stimulating the adrenal cortex indirectly, via the pituitary (26); this drug also failed to exert a differential effect upon the function of the two kidneys.

TABLE 3. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON RELATIVE WATER AND CHLORIDE REABSORPTION RATES FOR EACH KIDNEY—REPRESENTATIVE EXPERIMENTS

ANIMAL	TYPE OPERATION	PERIOD	PLASMA CHLORIDE mEq./lt.	MANNITOL U/P		$\dfrac{\text{MANNITOL U/P[1]}}{\text{CHLORIDE U/P}}$		COMMENT
				Rt.	Lt.	Rt.	Lt.	
Dog 6	Control	1	114.7	24.0	23.8	35.4	38.3	
		2	125.9	12.6	13.0	11.2	11.9	5% NaCl
		3	124.5	10.7	11.1	8.78	9.10	
Dog 3	Lt. adrenalectomy, chronic	1	111.7	19.0	14.1	31.1	19.1	
		2	112.1	30.2	16.7	44.4	17.1	
		3	122.2	24.4	14.7	23.2	12.1	5% NaCl
		4	129.8	17.2	12.1	12.8	8.84	
		5	129.3	17.0	14.6	11.9	10.2	
Dog 15	Lt. adrenalectomy, acute	1	112.5	41.9	40.4	32.5	33.1	
		2	113.1	44.8	46.4	37.0	42.9	Lt. adrenalectomy performed between periods 2 & 3
		3	117.7	55.2	33.8	120.	46.6	
		4	121.0	47.6	29.9	75.7	34.7	
		5	123.4	45.1	26.6	50.4	24.8	2% NaCl
		6	130.7	21.9	15.7	18.4	12.8	5% NaCl
Dog 8	Lt. splanchnic resection, chronic	1	112.7	49.1	16.3	70.9	11.5	
		2	111.9	79.6	18.5	165	12.5	
		3	113.2	48.8	22.5	47.4	15.1	2% NaCl
		4	113.8	59.8	28.9	77.5	21.6	
		5	114.9	45.1	29.4	40.9	21.3	Epinephrine
Dog 13	Lt. splanchnic resection, acute	1	113.3	35.0	34.9	61.4	50.2	
		2	114.5	32.3	31.1	39.7	33.9	Lt. splanchnic resection performed between periods 2 & 3
		3	116.5	41.3	14.7	72.8	11.2	
		4	124.4	21.5	7.90	22.3	6.21	2% NaCl
		5	140.2	12.4	5.22	11.2	4.14	5% NaCl
		6	137.7	15.6	5.24	14.3	4.06	
Dog 19	Bilateral splanchnic resection, lt. adrenalectomy	1	110.1	17.7	17.0	16.1	15.2	
		2	109.7	20.9	19.1	18.8	17.1	
		3	110.7	21.6	21.4	19.4	19.5	
		4	108.6	26.6	26.9	28.7	27.5	Epinephrine
		5	109.4	18.4	17.3	17.0	16.4	Epinephrine

[1] 'Chloride reabsorption ratio'.

Other effects of epinephrine. Epinephrine administered intravenously with the clearance solutions to *dog 14* (left adrenalectomy) tended to equalize the mannitol and PAH clearances in the two kidneys and abolish the marked disproportion in chloride and water excretion. A quantitatively smaller, but similar effect was seen in *dog 8* (left splanchnic resection, transection of sympathetic chain, table 1). However in 3 dogs (*18, 19* and *22*) in which there was no initial inequality in kidney function as regards the two sides despite left adrenalectomy with or without bilateral splanchnicectomy, epinephrine had little or no effect on the relative rates of excretion.

Tubular reabsorption of chloride and water. Our data permitted a calculation of the amount of chloride and water reabsorbed from the glomerular filtrate during each clearance period. On the assumption that mannitol is not absorbed from the tubules, we have employed the ratio $\dfrac{\text{urine mannitol concentration}}{\text{plasma mannitol concentration}}$, also designated

TABLE 4. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON THE AVERAGES OF RELATIVE WATER AND CHLORIDE REABSORPTION RATES FOR EACH KIDNEY

OPERATION	NO. ANIMALS	NO. PERIODS OF OBSERV.	MANNITOL U/P		$\dfrac{\text{MANNITOL U/P}}{\text{CHLORIDE U/P}}$	
			Rt.	Lt.	Rt.	Lt.
Control..........................	6	14	24.0	23.8	28.2	27.4
Lt. adrenalectomy..................	5	20	26.6	17.4	36.9	20.2
Lt. splanchnic resection..............	3	15	35.2	14.9	53.9	12.0
Bilateral splanchnic resection; left adrenalectomy......................	4	11	24.7	25.6	21.0	20.8

'Mannitol U/P', to represent the degree to which the glomerular filtrate is concentrated by the reabsorption of water.

Any substance which is partially reabsorbed (such as chloride, abbreviated 'Cl') is characterized by a U/P ratio less than the simultaneous mannitol U/P, the discrepancy being dependent on the amount reabsorbed. Hence, the $\dfrac{\text{man U/P}}{\text{Cl U/P}}$ ratio is always greater than 1.0; the higher the ratio $\dfrac{\text{man U/P}}{\text{Cl U/P}}$ (hereinafter termed 'chloride reabsorption ratio'), the greater the degree of reabsorption of chloride by the tubules. The values for this ratio determined simultaneously for the two kidneys in the same dog are shown in table 3; representative animals are chosen from experiments described above. The mannitol U/P ratios, indicative of the degree of water reabsorption, are shown for the same animals. In a control *dog 6*, and in the control periods of the acute experiments (*dogs 13* and *15*) both ratios on the two sides are nearly equal. They are also nearly equal in the bilaterally splanchnicectomized dog (*dog 19*) with one adrenal removed. In contrast, following either unilateral adrenalectomy or unilateral splanchnicectomy, the homolateral kidney invariably shows a lower chloride reabsorption ratio and mannitol U/P ratio than the control; this indicates a lesser degree of reabsorption of chloride and water in the homolateral kidney. Average values of the same ratios for all animals in the various groups studied are shown

in table 4; periods in which ureteral obstruction existed or drugs were administered are omitted.

<div align="center">DISCUSSION</div>

Explanation of increased excretion of water and chloride. From the observed values for urine volume and chloride content, serum chloride and mannitol clearance, it may be calculated that in 3 dogs (period 3, *dog 3*, table 1); periods 2, 3 and 4, *dog 4* (chronic unilateral adrenalectomy); and periods 4, 5 and 6, *dog 13* (table 1) the small increments in volume of glomerular filtrate observed after unilateral adrenalectomy or splanchnicectomy contributed water and/or chloride in amounts *less than* the extra amounts excreted. During all but two of these periods hypertonic solution of NaCl was administered. In the majority of periods of observation, the relative increment in glomerular filtrate observed to follow certain of the operative procedures provided amounts of chloride and water in excess of the extra amounts excreted by the homolateral kidney. However, this excess was not of great magnitude so that it was usually necessary to assume that an uncommonly large proportion of the 'extra' water and chloride filtered appeared in the urine with little reabsorption taking place in the tubules. That there is a relatively decreased tubular reabsorption of water and chloride following our operative procedures is also apparent from the decreased mannitol U/P and chloride reabsorption ratios presented in tables 3 and 4.

It is possible to interpret these findings as indicating that certain of our operative procedures had the specific effect of diminishing tubular reabsorption of chloride and water by direct action on the tubules. An alternative explanation is the possibility that the phenomenon is a consequence of the relatively increased glomerular filtration rate simultaneously observed. We have no information permitting a definite decision in this regard. In our own data we have noted no consistent relationship between small *spontaneous* changes in mannitol clearance in one kidney and the calculated chloride reabsorption ratio (table 3). However, in two experiments the relatively increased excretion of water and chloride in unilaterally adrenalectomized or splanchnicectomized dogs was reduced by the injection of epinephrine (e.g. *dog 8*, tables 1 and 3). The fact that the mannitol clearance in the homolateral kidney fell concomitantly suggested that the filtration rate and the degree of reabsorption of water and chloride were related.

On the other hand, certain other observations suggest that our operative procedures may have altered renal function by means other than increasing glomerular filtration rate. It was apparent that in the *intact, anesthetized* dog, the excretion of water and chloride usually was approximately proportional to the filtration rate; i.e., if the rate of filtration in the right kidney exceeded that in the left kidney by 5 per cent, the excretion of chloride and water by the right kidney exceeded that by the left to very roughly the same degree. However, in the *unilaterally* adrenalectomized or splanchnicectomized dog, the excretion of chloride and water was less closely proportional to the filtration rate; in these animals, an increase of 5 per cent in filtration rate in one kidney was occasionally accompanied by a relative increase in water and chloride excretion of 100 to 200 per cent (table 1).

The few observations on the effect of epinephrine in tending to equalize the

excretory activity of the two kidneys lend some support to the postulated specific influence of hormones from one adrenal gland upon the homolateral kidney. Unilateral adrenalectomy could produce its effects by abolishing the source of epinephrine, and unilateral splanchnicectomy could operate by interfering with liberation of epinephrine from the adrenal gland into the hypothetical adrenal-renal circulation. The fact that unilateral splanchnicectomy produces more striking changes in homolateral renal function than does adrenalectomy is evidence against the existence of a direct influence of secretions of the adrenal upon the homolateral kidney. Additional evidence was the absence of excretory changes in one animal (*dog 22*) following unilateral adrenalectomy.

Mechanisms producing increase in renal blood flow and filtration rate. Experimental interference with the transport of epinephrine to one kidney might be expected to result in vasodilatation in that organ and explain the observed increase in clearance of PAH and mannitol. It is more likely that any relative increase in renal blood flow in our experiments resulting either from adrenalectomy or splanchnicectomy was due to injury to the renal nerves rather than to interruption of any direct adrenal-renal vascular channel. The fact that the effects on the mannitol and PAH clearance following adrenalectomy were quantitatively less than those following splanchnic resection probably is an indication that adrenalectomy does not cause as extensive an interruption of nerve tracts leading to the kidney as does the latter operation. We interpret our failure to observe changes in excretion in *dog 22* (left adrenalectomy) as an indication that in this single instance we were able to remove the animal's left adrenal without injuring the renal vasomotor nerves. This experiment thus constitutes evidence that absence of one adrenal does not in itself influence homolateral excretion of chloride, water, mannitol, and PAH. Further proof is derived from the experiments on bilaterally splanchnicectomized animals; unilateral adrenalectomy performed on such animals did not result in a homolateral increase in renal clearances and chloride and water excretion even when the remaining intact adrenal was stimulated by adrenocorticotropic hormone (table 1). Presumably, the adrenalectomy did not injure more renal vasomotor nerves than had already been sectioned during splanchnicectomy.

Analysis of our data affords no conclusion as to the anatomical location of the vascular readjustments which must necessarily have taken place to cause the increase in renal blood flow and glomerular filtration rate subsequent to unilateral adrenalectomy or splanchnicectomy. There was observed no consistent change in filtration fraction and, hence, definite evidence of changed glomerular arteriolar tone is lacking.

Mannitol clearance as an index of glomerular filtration rate. Recently some doubt has been expressed that the mannitol clearance is a true measure of the glomerular filtration rate (7) in the dog. The mannitol clearance in this animal is reported to be lower than either the simultaneous inulin or creatinine clearance. Other workers (28, 29) using dissimilar methods for the analysis of mannitol, reported that in human beings the ratio of the mannitol clearance to that of inulin approximates 0.9. If tubular reabsorption of mannitol does occur, all calculations of glomerular filtration rate dependent on the value of the mannitol clearance are clearly in error to some

extent. For instance, a calculation of filtered chloride might be falsely low by as much as 10 per cent. However, in the analysis of our experiments, any error in the calculated filtration rate in one kidney will be largely compensated for by an error of the same magnitude on the opposite side.

SUMMARY AND CONCLUSIONS

1) The simultaneous renal excretions of chloride, water, mannitol and para-aminohippurate have been determined for each kidney in anesthetized dogs under the following conditions: *a*) control, *b*) after unilateral adrenalectomy, *c*) after unilateral splanchnicectomy and *d*) after bilateral splanchnicectomy and unilateral adrenalectomy.

2) Unilateral adrenalectomy in the dog usually results in markedly greater excretion of chloride and water by the homolateral kidney as compared to the control kidney. The excretion of mannitol and para-aminohippurate is usually slightly greater on the homolateral side.

3) Unilateral splanchnic resection, with or without concomitant partial sympathectomy, produces changes which are qualitatively similar but quantitatively greater than those observed after unilateral adrenalectomy.

4) In the bilaterally splanchnicectomized dog, unilateral adrenalectomy fails to augment relatively the excretion of mannitol, para-aminohippurate, chloride, or water by the homolateral kidney, even when the remaining intact adrenal cortex is stimulated by the administration of adrenocorticotropic hormone.

5) It is concluded that unilateral adrenalectomy specifically affected homolateral renal function only in so far as renal nerves were injured.

6) Following either unilateral adrenalectomy or unilateral splanchnic resection, the extra chloride and water excreted by the homolateral kidney occasionally cannot be accounted for by the accompanying slight increase in glomerular filtration rate. These two operative procedures both produce a relative decrease in the degree of tubular reabsorption of chloride. There is available insufficient evidence to permit a decision as to whether this decrease is due to a specific inhibition of tubular reabsorptive activity or to the increased rate of glomerular filtration.

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ELECTROGRAM OF TURTLE HEART STRIP IMMERSED IN A VOLUME CONDUCTOR

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THE electrical changes attending excitation and propagation can be understood only if the monophasic action potential curve is known.[1] The form of this curve may be determined experimentally and by mathematical analysis (1, 2). Theory indicates that the monophasic curve may be approximated by integrating the axial current curve (linear conductor); which, in turn, may be approximated by integrating the membrane current curve (volume conductor). The latter curve can be predicted semiquantitatively from potential theory (3, 4) and verified experimentally. By performing two successive integrations on this curve, one may determine the monophasic curve. If the relations postulated by the local circuit theory hold for heart muscle as well as for nerve, the calculated and the experimentally determined monophasic curves should be identical.

In demonstrating the interconvertibility of these curves we shall present a new graphical method for deriving the monophasic curve from the membrane current curve directly. Unfortunately, for reasons to be presented in the body of the paper, direct application of this method is not always practicable. However, since differentiation and integration are reciprocal operations, it follows that if we can select the monophasic curve which yields the experimentally derived membrane current curve, we shall have accomplished our purpose.

It is clear that such an analysis, no matter how successful, does not constitute a proof of our thesis, namely, that from the membrane current curve it is possible to determine the monophasic, and vice versa. It is necessary to demonstrate also that, when the form of the membrane current curve is drastically changed by controlled experimental procedures, the monophasic curve changes in the expected manner. This we shall do.

MEMBRANE CURRENT CURVE

Linear ventricular and auricular strips were deeply immersed in Ringer's solution. Unipolar recording was employed in conjunction with a Cambridge All-Electric Electrocardiograph. Non-polarizable electrodes were used; the remote electrode being at least 12 cm. from the exploring electrode and presumably negligibly affected by current generated by the contracting muscle. Stimulation of one

[1] Throughout we speak of the monophasic action potential curve as if it were the graphic representation of the time course of depolarization and repolarization of the cell membranes. We are not unaware that we are measuring potential differences in the external circuit and that the experimental record is only an approximation of the monophasic as thus defined.

end of the strip was effected by a make and break shock in rapid sequence through silver electrodes from a Harvard inductorium. There was no evidence of polarization due to the stimulating current.

Under these experimental conditions it is possible, as has been stated, to predict the form of the curve on the basis of potential theory. Provided potential gradients exist between the active region of the muscle and the resting or less active regions lying ahead and behind, local currents will be generated by the resting regions (sources) and discharge into the active region (sinks). A wave of excitation passing longitudinally down the muscle strip will give rise first to a diphasic deflection (positive and negative) associated with depolarization. Then, after an isoelectric period, there will be a diphasic deflection in the reverse order (negative and positive) associated with repolarization. The volume conductor curve, then, is polyphasic (fig. 1, A and B).

Fig. 1. TIME COURSE OF MEMBRANE CURRENT. *A*. Ventricular strip. *B*. Auricular strip. Spontaneous beats.
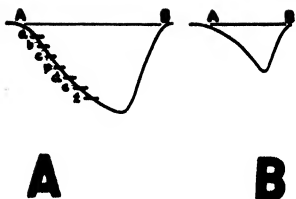
Fig. 2. CURVES ILLUSTRATING SPATIAL DISTRIBUTION of electrical density of auricular strip. *A*. Before applying mecholyl. *B*. After applying mecholyl.

The foregoing picture of excitation and recovery of heart muscle in a volume conductor has been given by Blair, Wedd and Young (5). Succinctly, their presentation involves two dipoles oppositely oriented and of equal strength passing under the recording electrode. But, as Macleod (6, 7) has pointed out, the repolarization process consists of a train of dipoles rather than just one. The distribution of the electrical density of this train in time, or space, is strictly related to the form of the descending limb of the monophasic curve. Our problem is to find a method for determining the intensities of polarization of the strip. Our solution is not explicit. Rather, it involves the representation of repolarization intensities by postulating a number of possible shapes for the descending limb of the monophasic curve. Graphical analysis of such curves yields experimentally verifiable membrane current curves.

Consider a strip of heart muscle, $A–B$, geometrically uniform and possessing homogeneous electrical properties in all its elements (fig. 2A). A wave of excitation is initiated at A and the potential changes are recorded at an intermediate point, p. (At p is a suction electrode which may be utilized to produce monophasic curves when desired.) Let the moment under consideration be the time when the wave of excitation has almost reached B. At this moment an observer at B looking backward sees

a contour for the total distribution of the electrical density of the strip from A to B. The membrane near B, not yet having been reached by the wave of excitation, bears the full complement of charges. Between f and B practically complete depolarization is assumed to exist. At e, however, recovery has begun and a few charges have been restored. Passing backwards to the point of stimulation, A, more and more charges have been restored. If the strip is long enough, 100 per cent repolarization will have taken place at A. We now make a fundamental assumption, namely, that the distribution of the charge density from f to A is given by the form of the descending limb of the monophasic curve recorded *in air*. Then the number of charges at c, p, and d (unit areas equidistant from each other) is such that during repolarization p acts as a source relative to d, and as a sink relative to c. The overall effect at p, however, is that of a sink with respect to the remote electrode (*vide infra*). This presentation neglects effects from b and d, a and f.

We may now plot the monophasic curve recorded in air at p on cross-section paper. The same curve is reproduced for point c to the left and, again, for d to the right of p. The horizontal distance between curves c and p, p and d, is the assumed conduction time from c to p, p to d, respectively. Since the wave of depolarization reaches c before p and p before d, the vertical distance at each instant between the ascending limbs of curves c and p is positive; that between p and d, negative. During repolarization, however, the vertical distances between the descending limbs of c and p become negative; those between p and d, positive. It is obvious, then, that the form of the membrane current curve can be approximated by *determining the difference in length at each instant of the verticals between curves c and p, p and d, respectively*, taking care to interpret the sign correctly.

The foregoing procedure may perhaps be made clearer by using a diagrammatic representation (fig. 3). Assuming that the repolarization process is a monotonic function, two kinds of monophasic curves are recognizable on the basis of the form of the descending limb. In one the descending limb is concave downwards; in the other, concave upwards (fig. 3, B and C, respectively). A limiting intermediate form is illustrated in figure 3A, in which the descending limb is a straight line.

The simplest case is represented in figure 3A. Actually, descending limbs which are linear in form may appear transiently in the conversion experimentally of the curve of figure 3B to that of figure 3C. Polyphasic curves similar in appearance to the graphical resultant (fig. 3D) of figure 3A have been recorded. Such curves must not, however, always be conceived of as related to a monophasic curve whose descending slope is a straight line. An alternative interpretation will be given further on.

The monophasic curve illustrated in figure 3B has the usual configuration of heart muscle becoming repolarized under normal physiological circumstances, in that the descending limb is concave towards the time axis. The resultant membrane current curve (fig. 3E) has the following characteristics. The peak of the inward flowing current phase of the impulse is greater in magnitude than that of the outward flowing current density. The segment between the depolarization and repolarization deflections is everywhere slightly negative rather than isoelectric. This segment then dips into a trough whose maximum negativity is less, in absolute value, than the value for the crest of the succeeding wave of positivity. Assum-

ing that the descending limb of the monophasic curve is logarithmic (a very rough approximation for ventricular muscle), the time interval between the peaks of the diphasic deflection due to depolarization is equal to that between the peaks of the diphasic deflection due to repolarization.

Finally, the monophasic curve illustrated in figure 3C is the prototype of an experimentally recorded curve to be discussed further on. The essential feature of this curve is that it has the general form characteristic of nerve becoming repolarized under normal physiological circumstances, in that the descending limb is convex towards the time axis. The resultant membrane current curve (fig. 3F) is triphasic.

EFFECT OF LACK OF ELECTRICAL HOMOGENEITY OF THE MUSCLE

The experimental membrane current curves resemble in general figure 3E, which is the resultant of figure 3B; compare with figure 1, A and B. Variations do occur. Thus the maximum value for the inward flowing current phase of the impulse is not always just slightly greater than that for the outward flowing current density. In some instances it is very much greater in value; in others, much less. We are unable
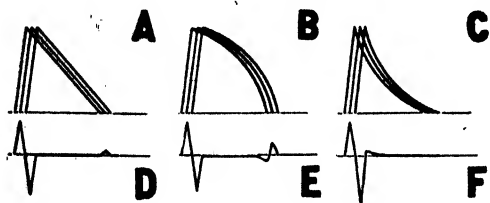
Fig. 3. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENTS, D, E and F, from the three types of curves, A, B and C, postulated for the monophasic action potential curve. See text. Ordinates in arbitrary units; those in A, B and C differing from those in D, E and F.

at present to explain this variability, though one or two obvious possibilities suggest themselves.

The segment connecting the depolarization and repolarization deflections is usually slightly below the isoelectric line, as predicted. Marked depression can be shown experimentally to be associated with effects due to nearby injured regions. Exaggerated elevation indicates injury under the electrode. By and large, our findings corroborate the studies of Eyster and Gilson (8).

More important for an understanding of conduction in heart muscle is a consideration of the time interval between the peaks of the diphasic depolarization deflection on the one hand and that between the peaks of the diphasic deflection due to repolarization. Our construction indicated that these time intervals should be equal, or nearly so. In our experimental curves the time interval between the peaks of the diphasic deflection of the impulse varies between one fifth to two thirds of the interval between the peaks of the diphasic deflection of the recovery process. The explanation for this discrepancy is that the tissue is not everywhere electrically homogeneous as was assumed in the theoretical treatment. It is well known that the elements of heart muscle show diverse rates of repolarization (9) and it is only because these rate differences are probably of a statistical character that the theoretically correct diphasic form is so frequently encountered experimentally.

We see, then, that the large preponderance of repolarization waves of the expected form is not only due to the sequence of depolarization of points c, p, and d

(fig. 2A), but is also contingent on the fact that near any point, p, picked at random, there will usually be areas of more rapid, and other areas of less rapid, repolarization. In several experiments we recorded not only from p, but also from a number of surrounding points. By this procedure, one can usually discover a point that yields a repolarization wave which is wholly positive (the region under the electrode becoming repolarized more quickly than, and therefore acting as a source for, closely adjacent regions) and another point that yields a purely negative repolarization wave (the region under the electrode becoming repolarized more slowly and behaving as a sink). In some records the repolarization wave is isoelectric, or almost so. This suggests that, provided the conduction velocity is constant (a valid assumption implicit throughout), the tissue under the exploring electrode, p, becomes repolarized just so much faster than that at c, but slower than that at d, that the effects cancel out. Finally, some records show a diphasic repolarization wave whose phases are reversed; i.e., in which the first phase is positive, the second negative. This may be

Fig. 4. ELECTROGRAMS ILLUSTRATING VARIATIONS in repolarization because of electrical non-homogeneity of the tissue. Compare with figure 1. *A* and *B*. Ventricular strips. *C* and *D*. Auricular strips. *S*, stimulus artifact.
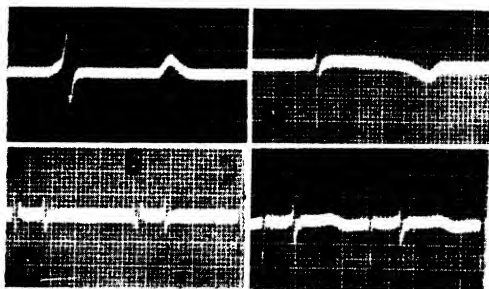
explained by assuming that initially the region under the electrode acts as a local anodal focus, but is soon dominated by a more rapidly repolarizing adjacent region. Experimental records illustrating these four types of repolarization waves are given in figure 4. It is, of course, possible to construct graphically corresponding membrane current curves by changing the duration of the properly chosen monophasic curves, c, p, or d, as each case demands.

That differences in the time course of repolarization of closely adjacent areas exist, and are responsible for the variations in the form of the deflections of the recovery process, may be more completely demonstrated by increasing the rate of stimulation and thereby reducing the cycle length. One of us has shown that this procedure effectively minimizes the *differences* in the time course of repolarization of the different muscle elements (10). A purely upright repolarization wave becomes diphasic as the cycle length is decreased and in figure 5 the diphasic character of this wave is seen to be accentuated. Furthermore, the interval between the peak of the negative phase and that of the positive phase of the repolarization wave is decreased. However, it is only when the time interval between the peaks of the diphasic depolarization deflection is unusually long (0.06 sec., instead of the more commonly recorded 0.01 to 0.03 sec. range) that this interval approaches that between the peaks of the diphasic repolarization deflection. We interpret this to mean that a residuum of electrical non-homogeneity is still present.

EFFECT ON THE MONOPHASIC CURVE OF EXPERIMENTALLY MODIFYING THE
MEMBRANE CURRENT CURVE

In figures 6A1 and 6B1 are shown typical monophasic records for ventricle and auricle respectively, recorded in air using the suction electrode. Below each experimental curve are shown: first, the diphasic axial current curves computed by numer-
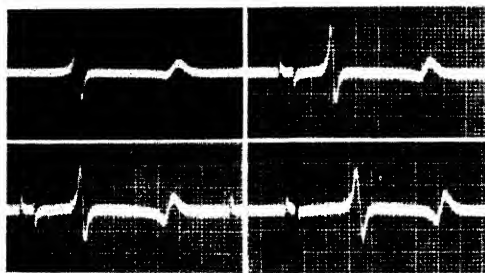
Fig. 5. ELECTROGRAMS ILLUSTRATING EFFECT of change of cycle length on repolarization. Cycle lengths: *A*. 8.6 sec.; *B*. 2.9 sec.; *C*. 2.1 sec.; *D*. 1.9 sec. Ventricular strip. *S*, stimulus artifact.
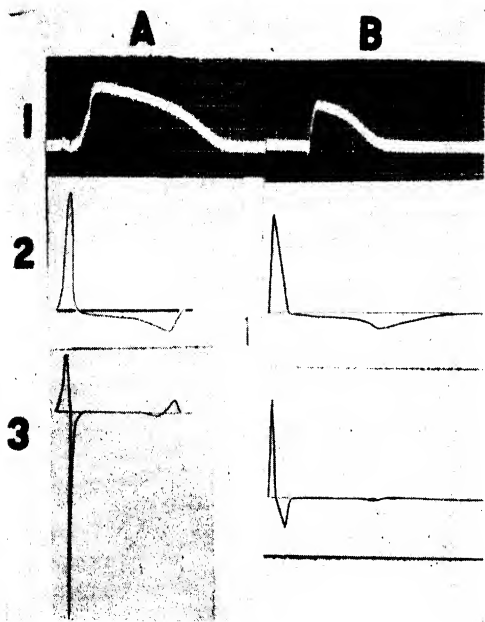
Fig. 6. A1 AND B1. MONOPHASIC ACTION POTENTIALS of ventricular and auricular strips, respectively, recorded in air with the suction electrode. Both show slight volume conductor effects. *S*, stimulus artifact. *A2* and *B2*. Time course of axial currents of ventricular and auricular strips, respectively, derived by numerical differentiation. *A3* and *B3*. Time course of membrane currents of ventricular and auricular strips, respectively, derived by numerical differentiation. Ordinates in arbitrary units and not comparable for different frames.

ical differentiation (figs. 6A2 and 6B2); and, secondly, the membrane current curves computed by numerical differentiation (figs. 6A3 and 6B3).

Copies of figures 6A1 and 6B1 are aligned for graphical analysis by our method in figures 7A1 and 7B1, respectively. The resultants are shown in figures 7A2 and 7B2.

It is instructive to compare the membrane current curves of figures 6A3 and 6B3, obtained by numerical differentiation, with those of figures 7A2 and 7B2 obtained by our graphical method and to compare both sets with the experimental records of figure 1. One sees that the resultants obtained by our graphical method

may provide a better basis for experimental prediction than those obtained by numerical differentiation. As a matter of fact, Cole and Curtis (11) have already pointed out the likelihood of lack of satisfactory prediction because of cumulative errors in the numerical calculation of the second derivative (and other reasons as well). As an aside, it may be of interest to note that our graphical method, when applied to the monophasic curve of Nitella, yields a maximum value of the inward current density which is definitely greater than that of the outward current density—a finding predicted by theory but not established by numerical differentiation. In general, it is quite clear that the relationships between the monophasic, di-, and polyphasic curves

Fig. 7. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENTS, *A2* and *B2*, from the monophasic action potentials, *A1* and *B1*. *A1* and *B1* are copies of the records of figure 6, *A1* and *B1*. Ordinates in arbitrary units and not comparable for different frames.
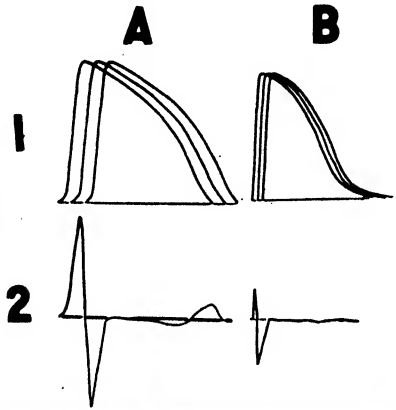
Fig. 8. *A*. TIME COURSE OF MEMBRANE CURRENT of auricular strip following application of mecholyl. *B* and *C*. Time course of axial currents of auricular strip before and after, respectively, applying mecholyl. *D*. Monophasic action potential of auricular strip following application of mecholyl. (Lead wires reversed from C.) *S*, stimulus artifact.
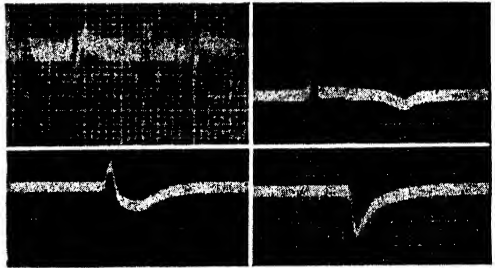
hold, so that the local circuit theory is valid for heart muscle under normal physiological conditions.

The validity of the theory would be greatly enhanced if it could be shown that the relationships hold under experimentally modified conditions. Accordingly we carried out some experiments on the effect of mecholyl on auricular strips immersed in a volume conductor. The effect of dilute solutions (c. 10^{-5}) of mecholyl is to convert the polyphasic form of the membrane current curve to a triphasic one like the resultant in figure 3F. The experimental record is shown in figure 8A.

Now, if our thesis is correct, namely, that, given a membrane current curve, it is possible to derive the appropriate monophasic curve, we should be able to record experimentally a monophasic curve for auricle treated with mecholyl which has the form shown in figure 3C. Linear strips in air were used. The normal diphasic action

potential curve was first recorded (fig. 8B; compare with fig. 6B2) and then mecholyl was placed on the tissue around one electrode. The diphasic curve underwent a series of changes, an example of which may be seen in figure 8C, until complete block supervened. The monophasic record is shown in figure 8D. Reoriented copies of the latter curve are arranged for graphical analysis in figure 9A. Obviously, experiment and theory are in good agreement. The graphical resultant is given in figure 9B; compare with figures 8A and 10B3.

A
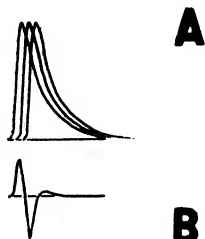
Fig. 9. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENT, *B*, from the monophasic action potential, *A*. *A* is a copy of the record of figure 8D. Compare figure 9B with figure 8A. Ordinates in arbitrary units, those in *A* differing from those in *B*.

B

A   **B**

1

2
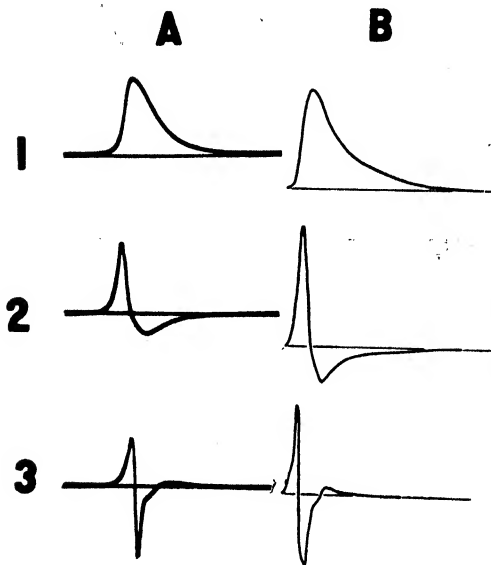
Fig. 10. COMPARISON OF TIME COURSE of monophasic action potentials, axial, and membrane currents of crab nerve (A1, 2, and 3, respectively) and of turtle auricular strip after applying mecholyl (B1, 2, and 3, respectively). Figures 10A1, 2, and 3 from figure 5 of Katz and Schmitt (18). Note that the time required for the inscription of the monophasic action potential of crab nerve is 4 msec.; of the turtle auricular strip, nearly 0.8 sec. Ordinates in arbitrary units and not comparable for different frames.

3

The profound change in the monophasic curve under the influence of mecholyl implies a modified distribution of the electrical charges on the auricular strip. We may now picture the contour for the distribution of the electrical density as in figure 2B. The principal change is the great decrease in length of the almost completely depolarized segment. This, we feel, is mainly due to an acceleration of the repolarization process. If the acceleration is sufficiently great, two effects may be expected. In the first place, currents derived from recovering or recovered regions will interfere at the active segment with currents originating from resting regions in front of the excitation wave. As a result, currents flowing out of the resting regions ahead of the impulse will meet with considerable resistance and be diminished in magnitude. Experimental evidence for this point of view is seen in the fact that for the auricular

strip treated with mecholyl the outward current density is very definitely less than that of the inward current density (fig. 8A). We venture to suggest that in nerve, normally, the repolarization dipole is advanced spatially, or temporally, so that its negative charge summates with that of the depolarization dipole. The magnitude of the positive charge of the depolarization dipole, as registered by the exploring electrode which it is approaching, will appear effectively lessened in magnitude.

The more obvious effect of the acceleration of recovery will be, as already stated, to decrease the length of the active segment. In terms of the dipole theory, this implies that the distance between the negative and positive charges of the repolarization dipole is decreased. One should bear in mind that in heart muscle the repolarization process is more accurately represented by postulating that the components of the repolarization dipole are more widely separated than those of the depolarization dipole (3).

Another, and equivalent, picture may be derived from an analogy with the reactions of ephapses (12, 13). When active and resting nerve fibers are contiguous over a sufficient length, the impulse generates currents which are, successively, anodal, cathodal, and anodal, in their effects on the resting fiber. Actually, the cathodal phase is double and appears as such in the normal heart muscle strip. The effect of mecholyl is to telescope these separate cathodal phases into one. The result is a picture like that of nerve (*vide infra*). Also, Arvanitaki (14) has shown for such double nerve preparations that in many of the geometrical arrangements the terminal anodal effect suppresses the active response of the resting fiber. In the case of the auricular strip treated with mecholyl, the activity of the depolarizing cathode may be thought of as if it were being aborted by the action of the terminal anode.

The foregoing presentation provides a point of departure from the conduction theories of Rashevsky (15) and Rushton (16). Their analyses postulate that the excitatory disturbance in effect leaves the tissue (nerve) permanently altered behind it. Electrical changes involved in recovery are assumed not to affect the electrical phenomena occurring in regions lying ahead of the excitation wave. In this connection see the paper of Offner, Weinberg and Young (17).

Having demonstrated that the local circuit theory of conduction in heart muscle is valid, and that our method of analysis of the electrical changes is self-consistent and capable of extension and prediction, we may be said to have accomplished the task set for ourselves. Before concluding, however, we should like to point out the interesting resemblance between the records of the electrical changes in auricular muscle, following the application of mecholyl, and those in normal nerve.

In figures 10A1, 2, and 3, we reproduce from the paper of Katz and Schmitt (18; page 478, fig. 5) the monophasic, diphasic, and membrane current curves, respectively, of crab nerve. The monophasic curve is obtained by electric integration of the diphasic; the membrane current curve by electric differentiation of the latter. In figures 10B1, 2, and 3, we present the monophasic, diphasic, and membrane current curves, respectively, of auricular muscle treated with mecholyl. The diphasic and membrane current curves are derived by successive numerical differentiation of the monophasic curve. Although the absolute temporal relationships between the two sets of curves are, of course, of an entirely different order of magnitude, the relative temporal relationships are strikingly similar.

SUMMARY

A new graphical method has been presented for deriving monophasic action potential curves from membrane current curves (volume conductor), and vice versa. The applicability of the method was tested by experimentally modifying the form of the membrane current curve and predicting the form of the monophasic curve. The prediction was verified by experiment. We conclude from this analysis that conduction in these heart muscle strips may be interpreted as though the tissue were a single fiber.

Variations that occur in the repolarization process are interpreted to mean that the tissue is not homogeneous in its electrical properties. Unless this phenomenon is absent, or recognized and adequately dealt with, the application of numerical methods of integration for deriving monophasic curves from membrane current curves may yield incorrect results. The difficulties can be resolved by assuming various forms of monophasic curves which, by trial and error, will give a fit between the graphical resultant and the experimental record.

Finally, from empirical considerations, we feel that any complete and adequate theory of conduction must take into account the effects of electrical changes in the recovery process upon those occurring ahead of the wave of excitation. Present theories fail to do this.

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EFFECTS OF CHANGES IN POSITION OF THE HEART OF THE CHICKEN ON THE ELECTROCARDIOGRAM[1,2]

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THE effects of changes in the position of mammalian hearts on the electro-cardiograms have been reported by a number of workers. This paper, to the writer's knowledge, represents the first report on the effects of position changes of the chicken heart upon the electrocardiogram.

METHODS

Before the heart was exposed, the electrocardiogram was taken with the bird lying on its back and anesthetized with pentobarbital sodium. The muscle from the sternum was then removed and the sternum and sternal ribs were cut away, leaving the coracoids and clavicle intact. As soon as the heart was exposed, its position was charted in relation to the limb leads. Needle electrodes were inserted in the muscles at the base of the wings and in the muscle of the left thigh. The relationship of the Lead lines to the long axis of the body of most birds was such that the apex of the heart had to be rotated from 80° to 90° to the left of the midline and approximately 60° to the right of the midline in order to be perpendicular to Lead lines *III* and II, respectively.

The heart was rotated only on its anterio-posterior axis and with the pericardium intact. The pericardium was freed of its attachments at the apical end and a piece of thread was tied to it. Thus the heart was rotated by rotating the pericardial sac. Attempts at rotating the heart with the pericardium removed usually resulted in injury currents and this method was abandoned.

The left ventricle of the chicken heart is about three times as large as the right ventricle (1) and the apex is curved slightly to the right. In rotating the apex to the left, the heart tends to rotate on its longitudinal axis more than when rotated to the right. This is discussed more fully later.

The electrocardiograms were recorded with a direct-writing, moving-coil galvanometer (Cardiotron) manufactured by the Electro-Physical Laboratories, New York City. The records of the three Leads were taken consecutively on the same instrument.

In calculating the electrical axes, the records were magnified and the amplitude recorded in 0.25 mm. In all cases the algebraic summation of amplitudes I and III equalled or approached closely those of II. In most cases the variation was not more than 0.5 mm.

RESULTS

Position of the Exposed Heart. The position of the heart of the chicken, as Lewis (1) has shown, is almost vertical. The hearts of the 7 birds studied were in the midline or slightly to the left side with the apex slightly to the right. In 2 of the birds the apex formed an angle of $+100°$ and in the remainder the angles were from $+95$ to $+90°$.

Normal ECG of the Chicken. Lewis (1), working with 2 chickens, described the electrocardiogram in that species. His description, except for Lead I, is the same as the writer's.

The ECG of Lead I of the normal bird is the most variable of the limb leads. Based upon serial ECG's of over 70 adult chickens (2), it was found that the ECG's of Lead I are of two general types with respect to configuration. Of the birds studied about 63 per cent exhibited one of the types and, in a few cases, the same bird exhibited both types in serial ECG's. A description of the two types follows:

Type A is characterized by an upright P, a very small, abortive upright R, followed usually by a relatively prominent S wave, and usually an upright T wave; the T in some cases, however, may be flat or isoelectric.

Type B is characterized by an upright P, followed by a relatively prominent upright R, and no S, or a small S wave. The T wave may be isoelectric, slightly positive or slightly negative. There are variations of these types, but in most cases R is more prominent than the S, or the reverse.

In Leads II and III, the R wave is very small or absent, while the S wave is prominent. The T wave is positive. P III usually is upright.

Changes in ECG Following Exposure of the Heart. One of the most prominent changes noted in the ECG of the exposed heart, as compared to that of the unexposed heart, was the tremendous increase in amplitude in all leads following exposure. The significance of the amplitude changes will be discussed in another report. The configuration of the ECG in Leads II and III was changed very little after exposure, except that in Lead III the P wave became inverted in most cases. Before exposure of the heart, the P wave was upright in 5 of the 7 birds. At that time, 5 of the birds exhibited a *Type B* Lead I, and 2 *Type A*. After exposure of the heart, 6 of the birds showed *Type B*. There were no other significant changes in contours of the ECG's except that usually the R or S was relatively more prominent than before the heart was exposed.

Effects of Rotation to the Left on RS and T. The S wave is normally the main ventricular deflection in Leads II and III and also in Lead I in some cases. In 5 of the birds studied, the main ventricular wave in I was an R (fig. 1). In determining the changes in the amplitude and electrical axes of ventricular depolarization, RS has been used as the most reliable indicator of that change. In all cases RS I and III before and after rotation equalled or closely approached RS II. Most of the hearts were rotated $45°$ and $80°$ to the left and some were also rotated $30°$ and $60°$. A summary of the results of all degrees of rotation on RS and T is shown in table 1.

RS. In 8 out of 12 observations of Lead I, following rotation, the RS's were less positive or more negative, as indicated in table 1. Two RS's were slightly more

positive, and one less negative after rotation. In one instance where the *RS* was positive, it showed no change after rotation.

In those birds where the main ventricular waves were upright in Lead I before rotation, they became, in most cases, negative after rotation; and the degree of
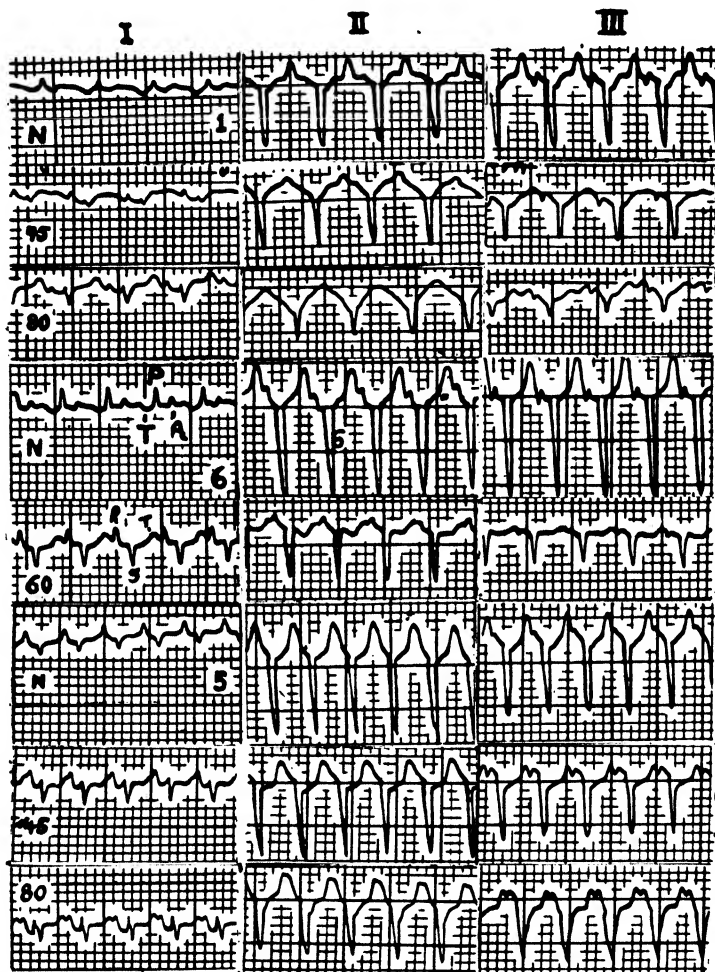

Fig. 1. ROTATION OF THE HEART on its anterio-posterior axis to the left. Leads I, II, and III for *birds 1, 5,* and *6* before rotation (N) and after different degrees of rotation. Standardization, 1 mv.

negativity, in most instances, increased with the degree of rotation (fig. 1, *birds 1* and *6*). In two cases, the main ventricular waves before rotation were negative; and the *R*'s were absent or very small. After rotation, *R* waves appeared or increased, and the *S* waves decreased (fig. 1, *bird 5*).

In Lead II, 9 of the 13 cases exhibited a decrease in negativity in *RS* and 4 showed an increase. In 3 of these the change was slight and probably not significant whereas one showed a significant change. In 10 of 13 cases for Lead III, the *RS*'s

exhibited a decrease in negativity, 2 showed a slight increase (probably not significant), and one showed no change. The variation in degree of change of *RS* in Leads II and III varied considerably; however, the degree of change in III was in most cases greater than in II, particularly with the higher degrees of rotation (fig.1).

Changes in electrical axes more clearly reflect the changes occurring in *RS* following rotation. The summary in table 2 shows that 11 of the *RS* axes were increased, and one was unchanged after rotation. The mean change in axes for all degrees of rotation was 11.7°, with a range of 0° to 32°. The maximum change was observed in *bird 4*. The axis for this bird before rotation to left was −68°, which was appreciably lower than the axes for the other birds.

The hearts of 5 of the birds were rotated 45° and 80° or 60° and 80° to the left. In all instances, except one, the changes in electrical axes were greater with the higher degree of rotation, but the degree of change was not directly proportional to the degree of rotation. The degree of change with 45° rotation, in most instances, and with

TABLE 1.   EFFECTS OF ROTATION OF HEART TO THE LEFT ON AMPLITUDE AND DIRECTION OF RS AND T. INCLUDES ALL DEGREES OF ROTATION

LEAD	NO. OF BIRDS	NO. OF ECG'S	MORE NEG. OR LESS POS.			LESS NEG. OR MORE POS.					NO CHANGE
			[1]—to[2]—	+ to —	+ to +	— to o	— to +	— to —	o to +	+ to +	
			Change in RS								
I	6	12	1	5	2	1	0	0	0	2	1
II	7	13	4	0	0	0	0	9	0	0	0
III	7	13	2	0	0	0	0	10	0	0	1
			Change in T								
I	6	12	0	0	0	0	4	2	2	3	1
II	7	13	0	0	13	0	0	0	0	0	0
III	7	13	0	0	13	0	0	0	0	0	0

[1] Sign of *RS* before rotation of heart.   [2] Sign of *RS* after rotation of heart.

higher degrees of rotation in some instances, was of low order and probably not significant.

T. In Lead I, 11 of the *T* waves were less negative or more positive after rotation, and one showed no change (table 1). The *T*'s which were negative before rotation usually decreased in negativity or became positive after rotation and the degree of change increased with the degree of rotation (fig. 1). In Leads II and III, the *T* wave in all of the observations showed a decrease in positivity, and the degree of change was greater in III, in most cases.

The electrical axes for all of the *T* waves showed a decrease after rotation to the left. The mean change in axes was +29°, with a range of +2 to +77° (table 2).

The more extensive change resulted from the greater degree of rotation, but the change was not proportional to the actual degree of rotation.

Effects of Rotation to the Right on RS and T.   *RS.* The effects of rotation to the right on *RS* and *T* were more consistent and pronounced than the effects of rotation to the left. A summary of the results is shown in table 3.

In Lead I, eight of the nine *RS*'s which were positive before rotation (*R* more prominent than *S*, or *S* absent) increased in that direction after rotation (fig. 2, *bird 7*). The degree of change was pronounced in most instances. Of the four *RS*'s which were negative before rotation three of these were only slightly so (*R* almost as prominent as *S*) and they decreased or became positive after rotation. Only one bird exhibited a prominent *S* wave before rotation and this became positive after rotation. In Lead II, 12 *RS*'s before rotation were negative and all of these decreased in amplitude after rotation. One *RS* which was positive before rotation

TABLE 2. INCREASE (I) OR DECREASE (D) IN ELECTRICAL AXES FOLLOWING DIFFERENT DEGREES OF ROTATION

BIRD NO.	AXES BEFORE ROTATION		DEGREE OF ROTATION LEFT				DEGREE OF ROTATION RIGHT					
	RS	T	45°		80°		45°		60°		80°	
			RS	T	RS	T	RS	T	RS	T	RS	T
1	−89°	+87	−5I	+2D	−23I	+17D	−32D	+25I	−45D	+75I		
2	−80	+96	−8I	+2I	−6I	+19D			−12D	+9I	−17D	+27I
3	−114	+109	−6I	+48D								
4	−68	+78	−32I	+43D			−14Dˣ	+78I				
5	−96	+85	−2I	+14D	−6I	+77D	−7D	+4I	−12D	+7I		
6	−85	+95	−27I¹	+36D¹	−19I	+50D			−4D	+13I	−13D	+20I
7	−80	+95	o	+9D	−7I	+30D	−9D	+8I	−28D	+42I	−28D	+44I

Summary: *RS*—11 increased, 1 no change;   12 decreased in angle; 12 increased in angle. *T*—12 decreased.

¹ 60° rotation.   ˣ = angle before rotation right (−16°). The axes of the other birds changed very little from those given.

TABLE 3. EFFECTS OF ROTATION OF HEART TO RIGHT ON AMPLITUDE AND DIRECTIONS OF RS AND T INCLUDES ALL DEGREES OF ROTATION

LEAD	NO. OF BIRDS	NO. OF ECG'S	RS MORE POS. OR LESS NEG.				NO CHANGE	T LESS POS. OR MORE NEG.				NO CHANGE
			− to o	− to −	− to +	+ to +	+ to +	− to −	+ to o	+ to −	+ to +	+ to +
I	6	13	2	1	1	8	1	10	2	1	0	0
II	6	13	0	12	0	1	0	0	0	2	11	0
III	6	13	0	13	0	0	0	0	0	0	10	3

increased slightly after rotation. The electrical axes for all of the *RS*'s decreased after rotation (table 2). The average decrease was −18.4°, with a range of −4° to −45°.

T. Changes. In Lead I, all of the *T*'s became less positive or more negative after rotation of the heart to the right (table 3). The *T* wave normally may be slightly negative or positive. Ten of the *T*'s which were negative before rotation became more negative after rotation (fig. 2). Of the three cases which were slightly positive before rotation, two became isoelectric, and one became negative after rotation.

In Lead II, all of the *T* waves were upright before rotation, and 11 showed a decrease in positivity after rotation (fig. 2). In two cases the decrease was from

positive to negative.   In Lead III, 10 of the *T*'s decreased in positivity and three showed no change after rotation.   The degree of change in II was, in all instances, greater than in III.

The electrical axes of all the *T* waves increased after rotation.   The average increase for all degrees of rotation was +20.3° with a range of 4° to 78°.   The degree of change in electrical axis for any given bird was greater with the higher degree of rotation, but there was considerable variation in the change between hearts receiving the same degree of rotation.   The degree of change, however, was not directly proportional to degree of rotation.   In most cases the actual change was less than the degree of rotation.   In two exceptional cases (*birds 1* and *4*) where the hearts were rotated 60° and 45°, the changes in axes were 75° and 78° respectively.
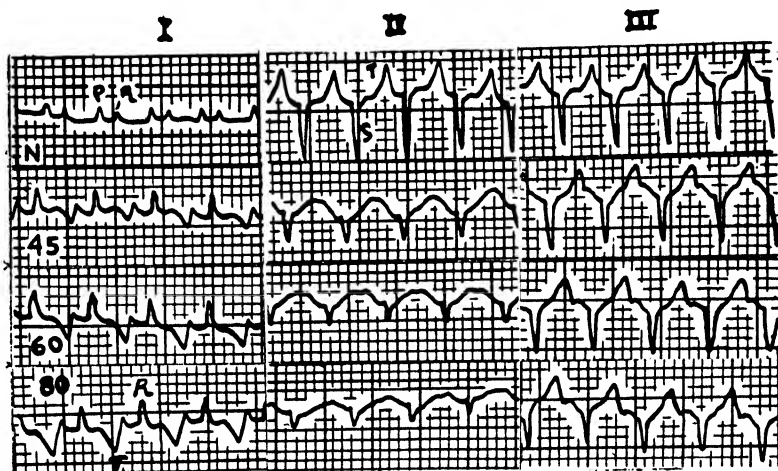
Fig. 2. ROTATION OF THE HEART on its anterio-posterior axis to the right.   Leads I, II, and III before rotation (N) and after different degrees of rotation.   Standardization, 1 mv.

DISCUSSION

The results obtained in this study are fairly consistent with expectations based upon Einthoven's theory.   In general, rotation of the heart to the left resulted in a great decrease in amplitude of S_3 and T_3.   The results obtained in Lead I depend upon the direction and configuration of *R*, *S*, and *T* before rotation.   In those cases where, in Lead I, *R* was relatively prominent (or *S* was absent) and the *T* wave was negative, rotation to the left resulted in a decrease in *R* and the appearance, or an increase of *S* and a positive *T* wave.   When a relatively prominent *S* and a small upright *T* were present in I, before rotation, the *S* decreased and *R* and *T* increased after rotation.   One of the birds studied was of this type.   In this case, the decrease in amplitude of S_3 was not so great as in the cases where an *R* wave was present in Lead I, as might be expected (fig. 1).

The changes, however, were not in direct proportion to the degree of rotation.   The electrical axes did not change in proportion to the shift in anatomic axes, even though the change, in most cases, was greater with the higher degree of rotation.   This was particularly true for the *RS* axes.   The change in the *T* axes with a given degree of rotation was greater than for *RS*.   The results of rotation to the right were more consistent and pronounced than in rotation to the left.   In the main,

rotation of the heart to the right resulted in a decrease in the amplitude of *S* II and III and of *T* II and III, and the decrease was greatest in *S* II and *T* II, in most cases.

In Lead I, the results obtained depended upon the direction and configuration of *R*, *S*, and *T* before rotation. Where *R* was relatively prominent in I and *T* was negative, rotation to the right resulted in an increased *R* and an increased negative *T*. These changes were in opposite direction to those obtained following rotation to the left. When an *S* and an upright *T* were present in Lead I (one case), before rotation, the *S* was replaced by an *R* and *T* became diphasic, but mainly negative with the higher degree of rotation.

The degree of change in electrical axes for *RS* was greater for rotation right, but was not directly proportional to the degree of rotation of the heart. The degree of change in the *T* axes was of the same magnitude for rotation to the right and to the left.

The results obtained for the chicken heart appear to be in closer agreement with Einthoven's theory than the results of rotation of the heart of dogs, as reported by Meek and Wilson (3), and others. Meek and Wilson showed, in rotating the heart of the dog on its anterio-posterior axis, that the heart was also rotated on its longitudinal axis, which affected the results obtained. This factor may have influenced the results obtained on the chicken heart, particularly in rotation to the left. The size and shape of the left ventricle is such that, in rotation to the left, the tendency for the heart to rotate also on its longitudinal axis is greater than in rotation to the right.

SUMMARY

The hearts of chickens were rotated on their anterio-posterior axes to the right and the left.

In rotation to the left there was a decrease in amplitude of *S* III and II and of *T* II and III, but the decrease was greatest in Lead III. In Lead I before rotation the ECG's of most of the birds exhibited a small *R* wave, no *S*, and usually a negative *T* wave. After rotation the *R* decreased or disappeared and was replaced by an *S* wave, and the *T* wave became positive. The electrical axes for *RS* increased in most instances after rotation and the *T* axes decreased. The degree of change in the axes was not directly proportional to the degree of rotation of the heart, but in most instances the change was greater with the higher degrees of rotation.

Rotation to the right resulted in a decrease in amplitude of *S* II and *S* III and of *T* II and *T* III, but the decrease was greatest in II. In Lead I, the *R* wave and the negative *T*, which were present in most birds before rotation, increased in amplitude after rotation. The electrical axes for *RS* decreased after rotation and the *T* axes increased. The degree of change in the *RS* axes was greater in rotation of the heart to the right than to the left, but the change was not directly proportional to the degree of rotation. In most instances, the change was greater with the higher degrees of rotation.

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STUDY OF SIMULTANEOUS RIGHT AND LEFT ATRIAL PRESSURE PULSES UNDER NORMAL AND EXPERIMENTALLY ALTERED CONDITIONS[1]

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THERE is indirect evidence that in the presence of an interatrial septal defect there is a transfer of blood from left to right atrium. This implies a greater pressure in the left atrium which has been directly demonstrated (1, 2). It has not been shown, however, whether such a pressure difference exists throughout the cardiac cycle or only at specific moments. As a first approach to the study of the hemodynamics of inter-atrial communications it is therefore important to know the normal relation between pulse contours, synchronicity of events and instantaneous pressure relations in the cavities of the two atria.

Although the right atrial pressure cycle and its variations is well-known, that of the left has received only a modest amount of attention, chiefly in connection with studies of pulmonary hemodynamics. The older observations consisting mostly of investigations carried out before the advent of modern optical methods of recording have been reviewed by Tigerstedt (3) and Wiggers (4). A few uncalibrated records of left atrial pressures obtained by modern optical methods have been published by Piper (5), Straub (6), Wiggers (7), Wiggers and Katz (8). Simultaneous recordings of right and left atrial pressures by adequate manometers are exceedingly few and none have been quantitated.

This investigation was carried out with the express purpose of comparing the basic effects which changes in blood flow have on right and left atrial pressures simultaneously recorded. Such studies can be made with greatest accuracy in open chest experiments in which the complicating effect of changes in intrathoracic pressure on venous pressures is abolished. In this way it is possible to establish a basis for comparison of results obtained when interatrial septal defects are experimentally produced. Furthermore, since the problem of hemodynamics in the pulmonary circuit seems destined to be reinvestigated by means of the right heart catheterization technique (9, 10), these observations should also be of use in interpreting the new information so obtained.

METHODS

Mongrel dogs of average size were adequately anesthetized with 3 mg/kg. of morphine subcutaneously and 180 to 200 mg/kg. of sodium barbital intravenously. Aortic and atrial pressures

were recorded by optical manometers of the Gregg design. The manometers used for aortic pressure had frequencies of at least 150 per second; those used to record atrial pressures were about 50 to 70 per second. There was no parallax between the various recording beams. Each manometer beam was calibrated in respect to its base line at the end of each record.

A tracheal cannula was inserted, the right carotid artery exposed and a femoral vein cannulated. Later, a cannula for recording aortic pressure was introduced via the right carotid artery so that the tip just reached the arch of the aorta. The approach to the atria was governed by the method of recording the atrial pressure contemplated. A mid-sternal approach with wide retraction of the chest wall was used when pressures were to be recorded via cannulae or semi-rigid catheters inserted through the azygos and/or pulmonary vein, or through the tips of the atrial appendages. In such cases the heart was suspended in a pericardial cradle, care being taken not to impede venous return. The mid-sternal approach was used occasionally when right atrial pressure was recorded by means of a sound passed down the external jugular vein. Usually, when the right jugular sound was employed the left atrium was exposed by resecting the third rib on the left side and left atrial pressure recorded by means of a cannula or catheter introduced via a pulmonary vein or the tip of the atrial appendage. Good records were obtained in a few experiments by resecting the third or fourth rib on each side and recording the atrial pressures through the tip of a 20-gauge hypodermic needle placed in the atrial cavity directly or through the tip of the appendage. Generally speaking, none of these methods has a marked advantage over the others. The rate and volume of artificial respiration was adjusted so that spontaneous respiratory movements just failed to occur. When taking records the respiration was halted for a period of 5 to 10 seconds in order to avoid artifacts and changes in hydrostatic level of the heart due to the inflation and deflation of the lungs.

RESULTS AND DISCUSSION

It was necessary in each case to determine the true pressure curve for each atrium and to minimize artifacts caused by intrinsic and extrinsic factors. The true forms of the atrial pressure curves were determined by taking numerous records with different positions and adjustments of the cannulae. Consistency in the form and pressure of a curve with the cannula in several different situations was the criterion of reliability. Records which contained artifacts that appeared to be caused by faulty position of the cannula, e.g., arterial impacts or occlusion during a part of the cycle, were discounted. Some artifacts, however, are present in *all* records. Movement of the heart, arterial impacts and heart sounds set up vibrations which are recorded by sensitive optical manometers. These are unavoidable in most cases if the manometers are of relatively great sensitivity. The analysis of form and instantaneous pressures in regions where they occur can therefore be only approximate.

Reliable records of simultaneous right and left atrial pressures were obtained from 30 animals before starting procedures such as rapid saline infusions, hemorrhage, or stimulation of nerves which could alter the normal relation between right and left atrial pressure. These records have been analyzed with the objective of determining whether any differences exist in the dynamics of blood flow in the right and left atria. Representative segments of normal records from six experiments are reproduced in figure 1 and discussed below.

I. *Pressure Pulse Contour.* The contour of the left atrial pressure pulse is fundamentally the same as the right, exhibiting the same waves but differing in amplitude. In general, the characteristic of the left atrial curve, as recorded by us, is the relatively greater elevation of the 'V' wave, the peak of which roughly coincides with the second heart sound. In most of our experiments the maximum pressure during the left atrial cycle occurred at this point. In this respect our curves differ from

some of those previously published. We attribute this to a more normal venous return than has heretofore been obtained in this type of experiment. The right atrial pressure pulses are typical and need no description.

II. *Synchronicity of Events.* Four easily identified points on the pressure curves were selected for comparison, these points being 1) the beginning of atrial systole, 2) the peak of atrial systole, 3) the end of atrial systole (in reality the beginning of

Fig. 1. SIMULTANEOUSLY RECORDED ATRIAL PRESSURE PULSES from 6 representative experiments. Top curve, left atrial pressure; middle curve, right atrial pressure; bottom curve, aortic pressure (A. P.).

ventricular isometric contraction, the so-called 'Z' point), and 4) the second heart sound artifact which we have called the 'V' point. Only records in which these four points could be identified without question on both right and left atrial curves were analyzed.

The results of this analysis are presented graphically in figure 2. No consistent pattern of asynchronicity exists. Right atrial systole may begin as much as 0.03 second before left, but in about 50 per cent of the cases the atrial systoles are simul-

taneous. Occasionally left atrial systole preceded right by 0.01 to 0.02 second. The peaks of atrial systoles may be synchronous or asynchronous in either direction and the precedence bears no relation to the order of precedence of the beginning of the atrial systoles. The end of left atrial systole as marked by the beginning of ventricular isometric contraction frequently precedes right, but this is a ventricular event rather than an atrial one. The appearance of the second heart sound artifact is simultaneous in both curves.
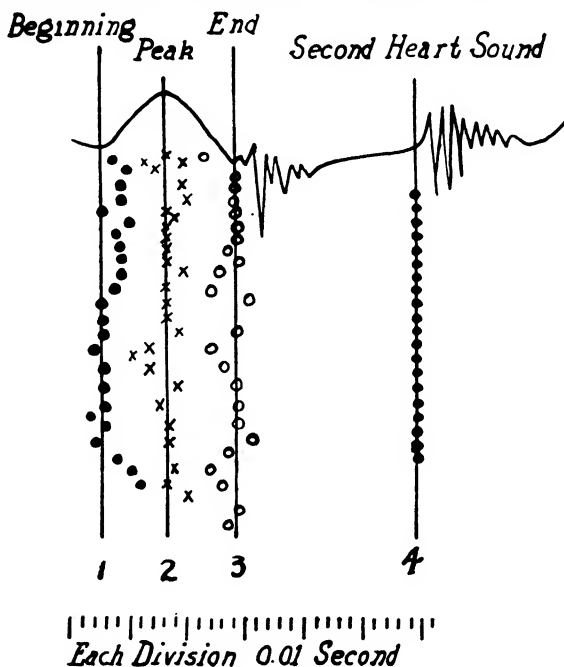
Fig. 2. TIME RELATION between comparable right and left atrial events.

III. *Pressure Relations Between Right and Left Atria.* An important objective of this investigation was to determine what hemodynamic gradients normally exist, or can be produced, between the two atria in the open chest animal that would be of importance in determining the direction and quantity of blood flow through a *theoretical* inter-atrial shunt. A comparison of simultaneously existing right and left atrial pressures throughout the cardiac cycle and under controlled experimental conditions gives us such information.

A. *'Normal' pressure relation.* The size and direction of the pressure gradient existing between the two atria from moment to moment was carefully measured in many experiments before instituting procedures which would experimentally alter the relation. By means of the coordirectograph described by Green (11) differential pressure curves were constructed by subtracting the right from the left atrial pressure curve. The six constructed curves reproduced in figure 3 are representative of all

types encountered.   Such curves show the direction and magnitude of the pressure gradient across the inter-atrial septum at each moment of the cycle.

In about 50 per cent of the cases (curves 1–3) the pressure gradient is from left to right atrium at all times during a single cycle.   Although the pressure gradient seldom exceeds 5 mm. Hg, it is evident that the direction of blood flow would have been from left to right atrium had an inter-atrial communication been present.   This observation adds weight to a clinical impression that such is the direction of flow through congenital shunts in man (1, 2).
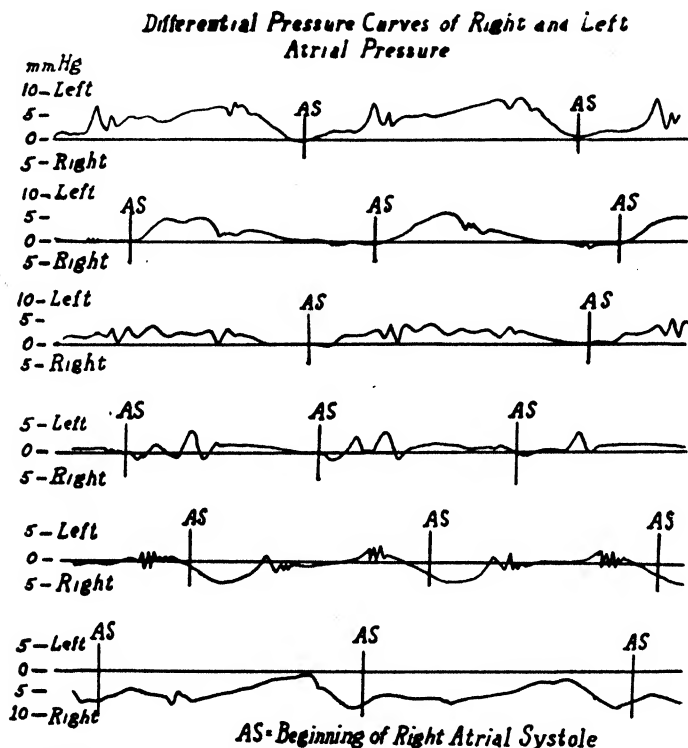
Fig. 3. CONSTRUCTED DIFFERENTIAL PRESSURE CURVES showing the moment to moment difference between right and left atrial pressure.   A-S, beginning of right atrial systole.

However, the pressure gradient across the inter-atrial septum is not always from left to right or even constant in direction.   In about 40 per cent of the cases of which curves 4 and 5, figure 3, represent the extremes, the gradient was predominantly from left to right, but reversed direction for a brief period (0.1–0.12 sec.) during some portion of the cycle.   The reversal of direction sometimes occurred during atrial systole and sometimes during diastole.   It did not appear to be associated with the degree of asynchronicity of the atrial events.   In most cases, however, the period during which the direction of the gradient was from right to left atrium was very short and the pressure differential very small (1–2 mm. Hg), so we are of the opinion that little blood would have been transferred from right to left atrium had an inter-atrial septal defect been present.

About one dog in 10 exhibited a right atrial pressure that was greater at all times during the cycle than left atrial pressure. A differential pressure curve of one such animal is illustrated by curve 6, figure 3. One of the striking things about such cases is the fact that when this situation is found the pressure differential is considerable, averaging 5 to 12 mm. Hg.

B. *Effect of infusions on atrial pressures.* It was our desire to alter the circulatory conditions in a number of ways in order to observe the effects on atrial pressures. One of the simplest methods of achieving this end is to increase venous return via an intravenous infusion.

TABLE I. SIMULTANEOUS RIGHT AND LEFT ATRIAL PRESSURES, IN MM. HG DURING INFUSION OF BLOOD

POINT	1		2		3		4	
ATRIAL PRESSURE	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
A. Control; A.P. 135/95, C.L. 0.42″	1.0	2.0	3.0	7.5	0.8	6.5	1.0	1.5
B. 50 cc. in; A.P. 155/122, C.L. 0.44″	2.5	6.4	4.0	7.0	2.0	9.0	2.0	4.1
C. 100 cc. in; A.P. 176/135, C.L. 0.46″	4.1	8.0	6.5	11.0	4.5	11.0	4.0	12.0
D. 150 cc. in; A.P. 193/145, C.L. 0.46″	5.5	11.0	9.0	15.0	7.2	15.5	7.0	18.0
TOTAL INCREASE	4.5	9.0	6.0	7.5	6.4	9.0	6.0	16.5

Pressures measured at pts. indicated in fig. 2. C.L. = Cycle length. Duration of infusion = 3½ min.

The effect of moderately rapid infusions of blood or saline into a femoral vein was studied first. The rate of infusion was usually 50 to 75 cc. per minute and the total volume varied between 100 and 500 cc. Simultaneous atrial pressures were recorded and the pressures measured at four different points during each cycle. These points, indicated in figure 2 were: 1) beginning of right atrial systole, 2) peak of right atrial systole, 3) end of right atrial systole, so called 'Z' point, and 4) just before appearance of second heart sound artifact, the 'V' point. Further reference will be made to these points by the appropriate number.

The change in atrial pressure relations as a result of infusion was remarkably constant in a series of 20 experiments. Therefore, the details of one of the best illustrates the typical result. Table 1 summarizes the data obtained from analysis of the optical records. By the time 50 cc. of blood had been infused the pressure in both atria was elevated at the four measured points, with a single exception (point 2 of the left atrial cycle). However, after 100 cc. had been infused the pressure had increased at all points. The striking feature in this case and all others is the fact that left atrial pressure increased more than right and by a considerable amount (see tabulation of total pressure change at each point). The greatest rise in atrial pressure, right or left, occurred invariably at point 4 on the left atrial pressure curve. The fact that the pressure at point 4 rose somewhat more rapidly, particularly after the first 50 cc. of infusion, strongly suggests that the rise of left atrial pressure was

due chiefly to an increase in left atrial inflow and not to back pressure effects occasioned by the rise of aortic pressure since the A-V valves were closed at this point.

We conclude on the basis of these experiments that a larger venous return would result in a greater pressure differential across the inter-atrial septum increasing the gradient from left to right. In the face of such a situation a larger quantity of blood would have been returned to the right circuit had a communication between the atria existed. Furthermore, it would appear that 'reversal effects' (i.e., a drop in systemic arterial oxygen saturation as a result of reversing the direction of flow through the shunt) would not have occurred as a result of infusion. A failure to decrease arterial oxygen saturation as a result of infusion has been reported in a human case with an inter-atrial shunt by Warren and co-workers (1).

The above experiments did not permit us to determine whether the elevation of right atrial pressure always preceded that of the left, or vice versa, since continuous records of infusion were not obtained. In order to clarify this point another series of experiments was performed. Brief rapid saline infusions at the rate of 10 to 50 cc. per second were made directly and alternately into the right and left atria via a right jugular sound or a cannula in the left atrial appendage. Atrial and aortic pressures were recorded continuously so that each record contained control, infusion, and recovery beats. Since the duration of infusion was usually not longer than 5 to 10 seconds it was possible to use a fast speed of the photokymograph, thus allowing the curves to be spread out and the pressure changes at various points of each consecutive cycle analyzed. Atrial pressures were measured at the previously indicated four points.

The changes observed with this type of infusion were consistent in a total of eight experiments. Therefore, the results of only one experiment are presented in detail in figure 4. The rate of infusion in this experiment was 18 cc. per second for a duration of a little over four seconds. As shown in figure 4, left atrial pressure increased considerably more than right at points 1, 2 and 4 during right arterial infusion. The increase appears to be parallel at point 3. Conclusions as to whether left atrial pressure begins to rise before right depend on which point of measurement one considers. Making allowances for random variation in the control pressure, the rise following the beginning of infusion is simultaneous at points 1, 2 and 3, but at point 4 a significant rise in left atrial pressure occurs before any significant increase in right pressure.

The reason for the simultaneous rise of both atrial pressures at points 1 and 2 shown on the first infusion beat is not clear. The increase in left atrial pressure apparently is not due to an instantaneous transmission through the pulmonary circuit. This could not be expected to elevate pressures other than at the V point. But as shown in figure 4 pressures are elevated at all points on the venous curve. This could be explained by a shift of the inter-atrial septum, but this appears to be excluded by the fact that the rise of pressures at the V point is greater on the left.

Infusion into the left atrium of the same dog at the same rate produces an entirely different effect as might be expected. Figure 4 reveals that left atrial pressure increases promptly and to a considerable extent on the first infusion beat. No significant rise in right atrial pressure had occurred at any of the measured points by the twelfth infusion beat, at which time the infusion was terminated. However, the

maximum left atrial pressure level did not exceed that finally achieved when saline was infused into the right atrium except at point 4. It should be noted, however, that control pressure at point 4 in the case of left atrial infusion was greater (4.7 mm. Hg) than the comparable control pressure in the previous case (3.5 mm. Hg). *The total pressure increase* in the two cases was, therefore, not greatly different.
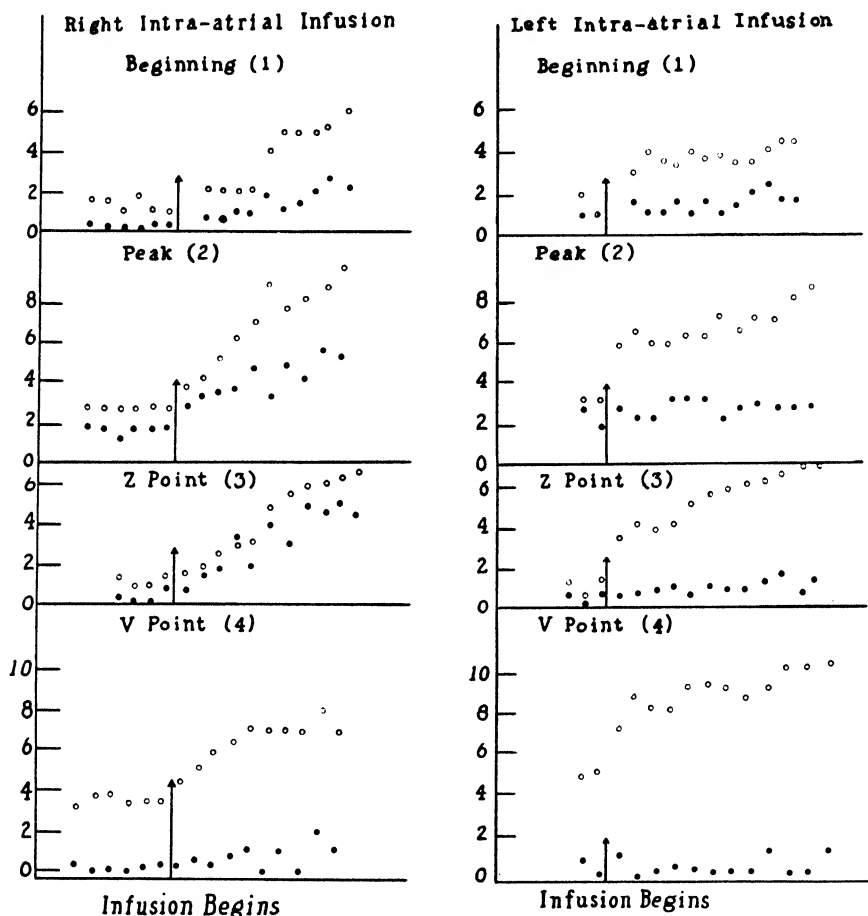
Fig. 4. ATRIAL PRESSURE CHANGES in successive cycles during rapid intraatrial saline infusion. Open circles, left atrial pressure at indicated points; solid circles, right atrial pressure at indicated points (see figure 2). Abscissae, mm. Hg; Ordinates, successive beats.

Little difference in the time at which aortic and pulse pressure began to increase was noted in this or other experiments when saline was infused into the right and left atria alternately. A comparison of systolic and diastolic pressures in consecutive beats during such a pair of infusions (table 2) shows that pulse pressure began to increase not later than the second infusion beat. Since infusion began in the right atrium during early diastole of the fourth control beat tabulated, the aortic pressure showed a significant rise on the succeeding beat. However, in the case of the left atrium, infusion began early in systole so a rise is not noted until the second infusion

beat.   For this reason it appears that there is little or no difference in the time at which aortic pressure increases.

The pulse pressure level increased somewhat faster in the case of left atrial infusion but systolic and diastolic pressure did not reach as high a level as when infusion was into the right atrium and pressures tended to fall before infusion was terminated. This is probably explained by the earlier arrival of the saline at the periphery, thus reducing peripheral resistance.   The change of pulse pressure in the early infusion beats, perhaps to the sixth beat (at which time the peripheral viscosity factor appears), indicates a marked increase in cardiac output as a result of the intra-atrial infusion.

The failure of right atrial pressure to rise when a very rapid saline infusion is made into the left atrium has several significant features.   In the first place it indicates that a rise in left atrial pressure does not reduce right cardiac output through back pressure effects.   In all probability right venous return is augmented during

TABLE 2. AORTIC PRESSURE CHANGES OCCURRING AS A RESULT OF INTRA-ATRIAL SALINE INFUSION. INFUSION RATE, 18 CC/MIN.   SAME CYCLES AS IN FIGURE 4.

	CONTROL				INFUSION BEATS										
CYCLE NO.......	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11
Syst..........	100	100	97	99	104	108	118	123	139	151	164	165	168	165	

Infusion of Right Atrium

Diast..........	71	71	68	—	74	77	85	91	99	107	115	115	115	110	
P. P...........	29	29	29	—	30	31	33	32	40	44	49	50	53	55	

Heart rate 162/min. except in beats 8, 9 and 10 where rate = 130/min.

Syst..........	98	103	102		108	125	136	140	143	145	147	146	140	138	140

Infusion of Left Atrium

Diast..........	70	72	73		79	90	98	99	100	100	99	95	94	88	87
P. P...........	28	31	29		29	35	38	41	43	45	48	51	46	50	53

No change in heart rate, 160/min.

the late infusion beats since the changes in pulse pressure indicate an increase in systolic discharge, although this is not reflected by any significant rise of right atrial pressure.   In the second place, if it is assumed that right heart output is not decreased by the greater left atrial pressure, then the pulmonary arterial and/or capillary bed must accommodate an increase in volume roughly equal to the infusion volume without backward pressure effects on the right heart.   The recent observations of Cournand (9) on the pulmonary bed capacity indicates that this is possible.   Thirdly, the magnitude of the atrial pressure rise, particularly at point (4), offers further evidence that an atrial septal shift is not involved in the simultaneous pressure increases noted when saline was infused into the right atrium.

The observation that right intra-atrial saline infusion produces a greater increase of pressure in the left atrium than in the right raised the question of the volume-elasticity characteristics of the atria and their associated great veins.   It is evident that the pressures at points 1, 2 and 3 tend to be determined by the venous inflow, by the capacity and elasticity of the atria, large veins and ventricles, since these

are freely communicating cavities. However, at point 4 the A-V valves are closed and the atrial pressures are functions of the inflow, capacity and elasticity of the atria and the great veins. On anatomical ground one would expect that the left atrio-venous system would have a greater volume-elasticity coefficient (more rigid) than the right since it has a smaller capacity, and the left atrial walls appear thicker (not necessarily so in the dog, but apparently so in the human (2)) and at equilibrium the inflow is the same into right and left heart. However, to the best of our knowledge differences in volume-elasticity relations of the two atria have never been demonstrated experimentally in the intact mammal. Our experiments offered an opportunity to at least study this in a semi-quantitative manner. Two different infusion rates were employed, right and left atria receiving an infusion alternately at each rate, sufficient time being allowed for recovery between observations. The increase in atrial pressure at the various points on the same side as the infusion were measured one second after the beginning of infusion and the values plotted against the infusion rate, as illustrated in figure 5. We now have a simple volume-pressure
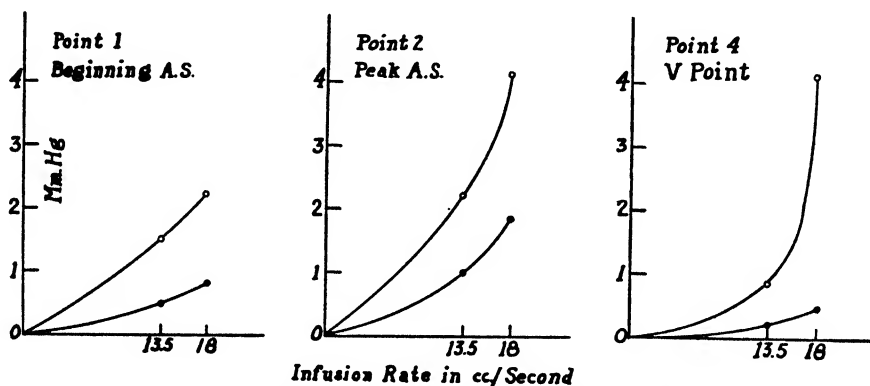
Fig. 5. QUASI-VOLUME-ELASTICITY CURVES of the right and left atrio-venous-ventricular cavities at various points of the atrial cycle.

relation graph in which the pressure change was measured and the volume is a function of the infusion rate (which was constant), plus the animal's own venous return which was assumed to be constant. It is obvious that this is a crude demonstration and that the volume-pressure relation cannot be given in quantitative terms. It does indicate, however, that the left heart is a less elastic structure than the right since the same volume inflow results in a greater left atrial pressure. We are particularly impressed with the restricted distensibility of the left atrio-venous system and, conversely, with the apparently great ability of the right atrio-venous system to store blood without a significant increase in pressure.

C. *Effect of hemorrhage on simultaneously recorded atrial pressures.* Dogs were bled from the femoral artery at the rate of 50 cc. per minute. Both right and left atrial pressures were observed to decrease simultaneously, but the rate of decline was faster for left atrial pressure than for right. The data from one hemorrhage experiment have been assembled in table 3. Atrial pressures were measured at the four usual points. Note that at each point the total decrease in pressure is greater in the left than in the right atrium.

The greater decrease in left atrial pressure than in right will of course decrease the pressure gradient from left to right across the interatrial septum, and if an inter-atrial shunt were present it would decrease the rate of flow into the right atrium from the left. However, the direction of the gradient would not be reversed until the animal had progressed to a moribund state.

D. *Effect of increased peripheral resistance on atrial pressures.* An increase in peripheral resistance in the intact animal produces a complex series of circulatory adjustments. Theoretically, right venous return should decrease due to vasoconstriction thus reducing right venous return, atrial pressure, and right heart output, resulting ultimately in decreased left heart output. Such a sequence is not observed

TABLE 3. CHANGES IN LEFT AND RIGHT ATRIAL PRESSURES RESULTING FROM HEMORRHAGE. PRESSURES MEASURED AT POINTS INDICATED IN FIGURE 2

POINT		1		2		3		4		CYCLE LENGTH
Atrial pressure		mm. Hg		mm. Hg		mm. Hg		mm. Hg		seconds
Control										
A.P. 135/101	Rt..............	6.0		9.5		2.0		5.2		0.34
	Lt..............		7.3		8.0		3.5		8.0	
50 cc. out										
A.P. 124/90	Rt..............	5.5		9.0		1.7		5.0		0.34
	Lt..............		6.8		8.2		3.0		6.8	
150 cc. out										
A.P. 97/67	Rt..............	4.8		8.0		1.5		4.5		0.34
	Lt..............		5.5		6.3		3.0		4.5	
250 cc. out										
A.P. 57/35	Rt..............	4.5		7.0		1.8		4.0		0.38
	Lt..............		4.0		4.0		1.0		4.5	
TOTAL DEC.	Rt..............	−1.5		−2.5		−0.2		−1.2		
	Lt..............		−3.3		−4.0		−2.5		−3.5	

in the intact animal, however, because the emptying of blood reservoirs and the increased arterial pressure serve to maintain or even increase right venous return and cardiac output may increase (8).

Since increasing peripheral resistance results in such profound circulatory adjustments, it was desirable for our purposes to observe the effect on simultaneously recorded atrial pressures. The increase in peripheral resistance was achieved by stimulation of the central end of the divided left vagus by means of an electrodyne stimulator. Stimulation was continued until the aortic pressure reached a plateau and began to fall.

Segments of a record from one such experiment are reproduced in figure 6. After 18 seconds of left central vagus stimulation (segment *B*) aortic pressure was elevated, but there was no significant change in left atrial pressure at any of the measured points. Right atrial pressure increased only slightly, the change being 0.5, 2.2, 0.9,

and o.5 mm. Hg at points 1, 2, 3, and 4 respectively. After 28 seconds of stimulation (segment *C*) aortic pressure reached a maximum with left atrial pressure increasing on an average of 7 or 8 mm. Hg at the respective points. Right atrial pressure showed no significant change from the preceding record, nor did it change appreciably with continued stimulation (95 sec., segment *D*). Following the cessation of stimulation (segment *E*), aortic and left atrial pressures decreased toward control level, but were still somewhat higher than before.

It is obvious that complex circulatory adjustment resulted from the increase in peripheral resistance occasioned by the vagal stimulation. Questions arise as to whether cardiac output increased, whether systemic venous return increased, as to the effect of the slight changes in heart rate on atrial pressures and a myriad of other aspects of the compensation. However, the analysis of these questions is beyond
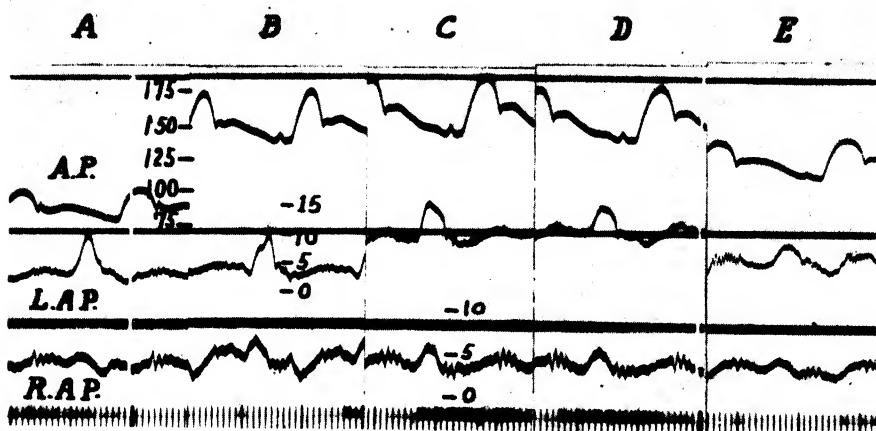
Fig. 6. EFFECT OF CENTRAL VAGAL STIMULATION on right and left atrial pressures simultaneously recorded. A-P, aortic pressure curve; L-A-P, right atrial pressure curve; A, control; B, after 18 seconds of stimulation; C, after 28 seconds; D, after 38 seconds; E, recovery (incomplete).

the scope of this paper. The significant point here is that left atrial pressure increased far more than right, thus increasing the gradient from left to right atrium across the interatrial septum. Had an interatrial shunt been present a rather large quantity of blood would have undoubtedly passed back into the right heart circuit.

E. *Effect of lung inflation on atrial pressures in the open chest dog.* The artificial respiratory rate in the various experiments ranged from 10 to 20 cycles per minute. Some variations of atrial pressure with respiration were observed in all experiments, but the amplitude of these variations was surprisingly small. Both right and left atrial pressure increased slightly with the positive pressure inflation and decreased with the passive deflation. This effect of respiration was observed consistently.

Since the respiratory cycles were rather short in most experiments (ca. 3 sec.), the effect of inspiration and expiration on atrial pressures was intensified by maintaining the lungs in a state of inflation or deflation for a period of five or six seconds and observing the changes in atrial pressure after a new equilibrium had been established. As compared to the pressures during expiration, maintained inspiration invariably caused both right and left atrial pressure to increase, the right much more

than the left, and aortic pressure decreased 10 to 20 mm. Hg with pulse pressure increasing only slightly.   Segments of records made during expiration and maintained inspiration are reproduced in figure 7.

The rise in right atrial pressure as shown in figure 7 is usually greater than that of the left.   In the case illustrated, right atrial pressure at points 1, 2, 3, and 4 during expiration were 3.8, 6.4, 4.5, and 4.0 mm. Hg respectively, but during maintained inspiration the values were 7.8, 11.2, 7.9, and 9.0 mm. Hg.   Comparable left atrial pressures during expiration were 5.0, 8.0, 7.0, and 9.0 mm. Hg and during maintained inspiration 7.2, 11.2, 9.0, and 10.8 mm. Hg.   From these experiments it appears theoretically possible that a maintained positive pressure inspiration in an open chest
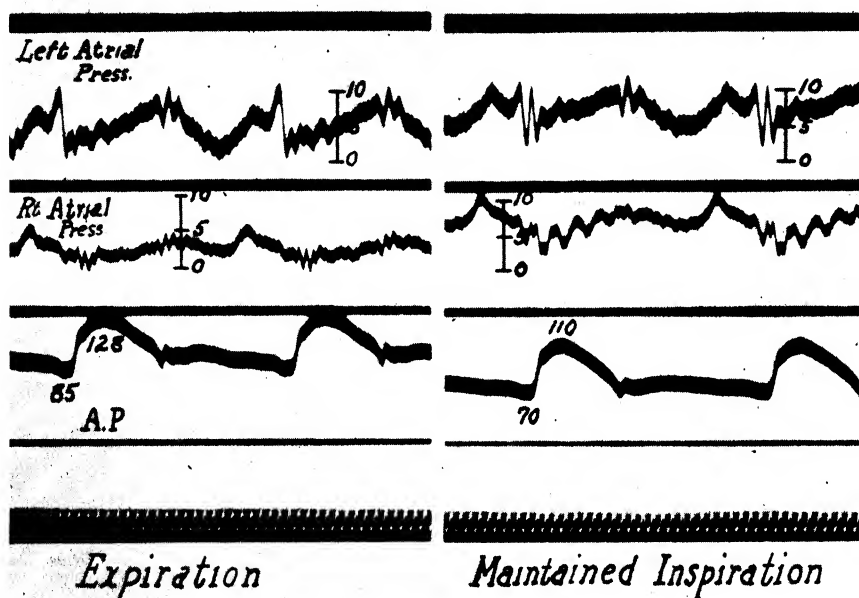
Fig. 7. EFFECT OF LUNG INFLATION (open chest) on right and left atrial pressures simultaneously recorded.

dog would tend to reduce or reverse the flow of blood through an interatrial septal defect.   In some instances the right pressure was augmented considerably more during inspiration than in the case used for illustration.

The results of this investigation confirm the numerous reports that left atrial pressure exceeds the right in the dog, although exceptions were noted.   However, the question could be asked whether the difference is apparent or real.   Since the right atrium is above and partially overlying the left when the dog is in the supine position there is a possibility that the left atrial pressure is greater because of a difference in the hydrostatic level of the atria.

We have demonstrated to our satisfaction that the difference is real by the expedient of filling the open thorax with saline to a depth that covered the beating heart and recording simultaneous atrial pressures while using the upper level of the saline as a common pressure reference level.   This obviated any difference in hydro-

static level. The pressure difference existing between the atria was unaffected by this procedure. The greater left atrial pressure must result from a more restricted distensibility of the left atriovenous system and not to gravitational factors as suggested by Uhley (12). When one considers that at equilibrium right and left atrial inflows are equal the finding of a real pressure difference between the atrial pressures confirms a prediction which might have been made on the basis of the volume-elasticity curves previously presented.

Proceeding on the assumption that the left atriovenous system has a greater volume-elasticity coefficient than the right it can also be predicted that a wider range of pressure fluctuations would be encountered in the left atrium. Re-examination of the curves and data reveals that this is true. Left atrial pulse pressure is almost always greater due chiefly to the relatively high pressure created by the atrial inflow during the time the A-V valves are closed. This is in accord with the thesis that the left atriovenous system has a restricted distensibility. The greater fluctuation of left atrial pressure is also demonstrated by alterations in the rate of atrial inflow. Increased inflow (infusion and probably stimulation of central vagus) invariably resulted in a greater elevation of left pressure, whereas decreased inflow (hemorrhage) lowered left pressure more than right. It has been commonly assumed that the range of pressure change in the left atrium parallels that of the right. It is apparent that this assumption is not entirely justified. Care must be exercised, therefore, when making calculations involving pulmonary arteriovenous pressure differences in which the venous pressure change is estimated. There is grave danger that the left atrial pressure change may be underestimated.

If atrial inflow is relatively constant the pressure within the atrial cavity during a single cycle will vary in accordance with the outflow (ventricular filling) and the volume-elasticity characteristics of the atrium. Changes in atrial inflow are, therefore, difficult to judge unless the degree of ventricular filling is known. This difficulty can be empirically obviated if one infers changes in atrial inflow from the change in atrial pressure only at the beginning or end of ventricular filling—the V or Z point. At these stages the outflow from the atrium is zero or negligible and pressure is relatively high. The atrial volume elasticity coefficient is greater and hence an increase in volume inflow will result in a more marked pressure elevation. For this reason it seems preferable to estimate changes in venous return on the basis of a pressure change at a single point of the atrial cycle rather than from changes in mean atrial pressure alone. It has been our experience that the major deformation of atrial pressure curves by artifacts occurs during the time of ventricular systole. When only mean atrial pressures are calculated such deformation may be sufficient to mask significant changes in atrial pressure during other portions of the cycle. This is particularly important when dealing with right atrial pressure, since the volume elasticity coefficient is low and only a slight change in pressure results from a relatively large change in inflow.

Two independent investigations (1, 2) have shown left atrial pressure to be greater than right in the presence of an interatrial septal defect in human subjects. Unfortunately, normal left atrial pressures have not been determined in the human for obvious reasons. Therefore, it is difficult to say whether or not these high left atrial pressures should be considered pathological. However, the observation of a

left to right atrial pressure differential in the open-chest dog is tenuous evidence that the high left atrial pressure observed in humans with interatrial septal defects is not entirely pathological. An incomplete series of experiments on dogs in which inter-atrial septal defects have been experimentally produced indicate that the pressure differential continues to exist. The observation that increased venous return always augments left atrial pressure to a greater extent than right negates the possibility of using infusion as a means of decreasing the pressure difference between the atria. This is a possible explanation for the failure of rapid infusion to reverse the direction of flow through an interatrial septal defect in a human patient as has been reported (1).

SUMMARY

Right and left atrial pressure pulses have been simultaneously recorded in thirty open chest dogs. No consistent synchronicity or asynchronicity between comparable events in the two atria was observed. Left arterial pressure is usually greater than right during the entire atrial cycle, but the direction of the pressure gradient across the interatrial septum may reverse during a cycle.

The pressure relation between the atria was also studied under various circulatory conditions. Rapid blood or saline infusion always increased left atrial pressure more than right, as did central vagus stimulation. Hemorrhage decreased left atrial pressure more than right. The existence of a pressure gradient and the variations thereof is due to a difference between the volume elasticity characteristics of the atria, the left atrio-venous system being less distensible than the right.

Changes in atrial inflow are most correctly inferred from changes in atrial pressure just at the beginning or end of ventricular filling. The atrial volume elasticity coefficient is greatest at these points since the A-V valves are closed and a relatively small change in inflow will produce a relatively large change of atrial pressure.

The greater volume elasticity coefficient of the left atriovenous system accounts for the greater left atrial pressures found in cases with interatrial septal defects and indicates that the elevated left atrial pressure is not entirely pathological. Furthermore, the more restricted distensibility of the left atrium accounts for the failure of infusions to reverse the direction of blood flow through the shunt.

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DYNAMIC EFFECT OF INSPIRATION ON THE SIMULTANEOUS STROKE VOLUMES OF THE RIGHT AND LEFT VENTRICLES[1]

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THE dynamic effects of inspiration and expiration on the systolic discharge of the right and left ventricles have been frequently studied with contradictory conclusions. According to one viewpoint, the act of inspiration increases venous return and elevates effective right atrial pressures both in animals (1) and in man (2). It is believed that this causes a larger stroke volume of the right ventricle (3, 4). However, the larger discharge is more than accommodated by the increased capacity of the pulmonary bed which results from lung inflation. The increased capacity of the pulmonary vascular bed during inspiration is believed to effect an accumulation of blood in the lungs and to reduce the flow to the left heart. Consequently, effective left atrial pressure decreases, left ventricular discharge diminishes, and aortic pressure declines.

According to another view, inspiration is without effect on venous return or effective auricular pressure. Consequently the stroke volume of the right ventricle remains nearly constant. The elevation in effective pulmonary arterial pressure is attributed to an increase in pulmonary resistance brought about by lung inflation. In support of this view are the recent observations of Duomarco (5) that similar rise of pulmonary arterial pressure occurs during lung inflation when the output of the right heart is kept constant by artificial means. Furthermore, according to Visscher (6), the increased capacity of the pulmonary vessels observed when the lungs are inflated may not take place when the head of pressure as well as the lungs are inclosed in a chamber in which the varying negative pressure can be reduced. Under these more physiologically correct conditions the pulmonary vascular bed is said to decrease and the resistance to increase. The inspiratory decline of aortic pressure recorded against a constant atmospheric pressure is generally interpreted as a transmission of the decreasing intrathoracic pressure to the aorta.

METHODS

This investigation was undertaken to evaluate simultaneously changes in right ventricular stroke volume by meticulous measurement of effective right atrial pressure and alterations in the stroke volume of the left ventricle by a careful study of aortic pressure pulses. Observations were made during natural breathing and deep breathing following vagotomy in dogs. Dogs were anesthetized with morphine and

sodium barbital.   Aortic pressure was recorded by means of a Gregg manometer connected to a sound introduced through the carotid artery and right atrial pressure by a similar connection to a jugular sound.   Since intrathoracic pressure apparently varies in different portions of the chest (7, 8), an intrathoracic cannula connected to a Frank segment capsule which could be standardized against a water manometer was thrust through the chest wall in the third left interspace so that its tip lay in close proximity both to the tip of the right auricle and root of the aorta.   As indicated in figure 1, the technique employed was that of Wiggers, Levy, and Graham (8) who compared pressure relations in several regions of the chest, but not in the space
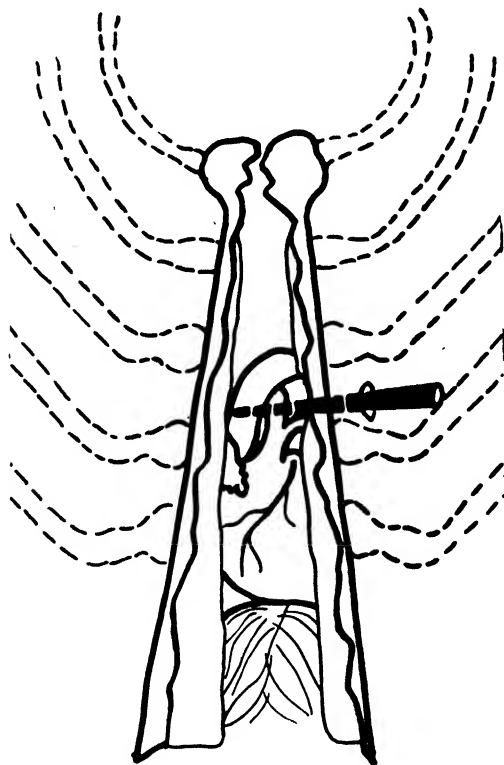
Fig. 1. POSITION OF INTRATHORACIC CANNULA in relation to right auricle and aorta.

surrounding the right atrium and aorta.   Once in position, a cavity was created by simply rotating the flat-tipped cannula in various directions until a smoothly recorded curve with superimposed variations was obtained.   The location of the cannula was verified by post-mortem examination in all experiments.   In this way the true pressure differences between the interior and exterior of the right atrium and aorta could be determined at every moment of the cardiac and respiratory cycles.

RESULTS

Illustrative records are shown in figure 2.   Records A and B were obtained from naturally breathing animals; records C and D, during prolonged deep inspiration resulting from vagotomy.   It will be seen at a glance that the cardiac variations of

intrathoracic pressure in the space around the right atrium and aorta in acceptable records are characterized by a significant diminution during atrial contraction and a slight elevation during ventricular systole. (Fig. 2A, marked *1* and *2*). Therefore, in determing effective atrial and aortic pressures at any point of the cardiac cycle simultaneous values of intracardiac pressures must be utilized.
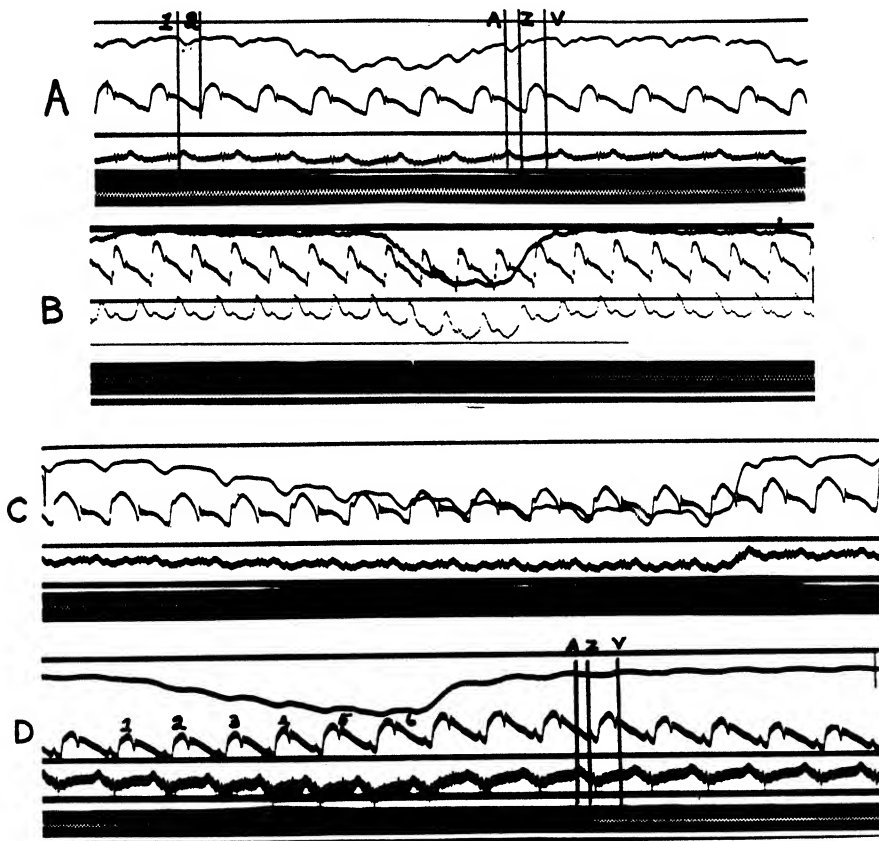
Fig. 2. FOUR RECORDS showing successive changes in intrathoracic, aortic, and right atrial pressures during inspiration and expiration; *A* and *B* during natural breathing; *B* and *C* after vagotomy. Time in .02 second. About ⅔ actual size. *A*, lines 1 and 2 showing changes of pressure around atrium and aorta due to atrial and ventricular contractions. Lines A, Z, and V show points at which corresponding pressures were measured at peak of atrial systole, at onset of ventricular contraction and at beginning of ventricular filling.

In order to obtain a complete analysis of effective pressure variations the atrial component was measured at three points of the cardiac cycle, viz., 1) the peak of atrial systole, 2) the beginning of ventricular isometric contraction and 3) the point of maximal atrial volume. These points are marked *A*, *Z*, and *V*, respectively, on one of the records of figure 2. Simultaneous points on the intrathoracic pressure curve were then measured and the effective pressure calculated as the algebraic difference of these two values. Such measurements of records from 10 experiments yielded the following results. In three, atrial pressure showed no measurable difference;

in three it decreased a trifle more during inspiration (ca. 1 mm. Hg) than extracardiac pressure, i.e., effective pressure was reduced a trifle.   The reduction in effective pres-
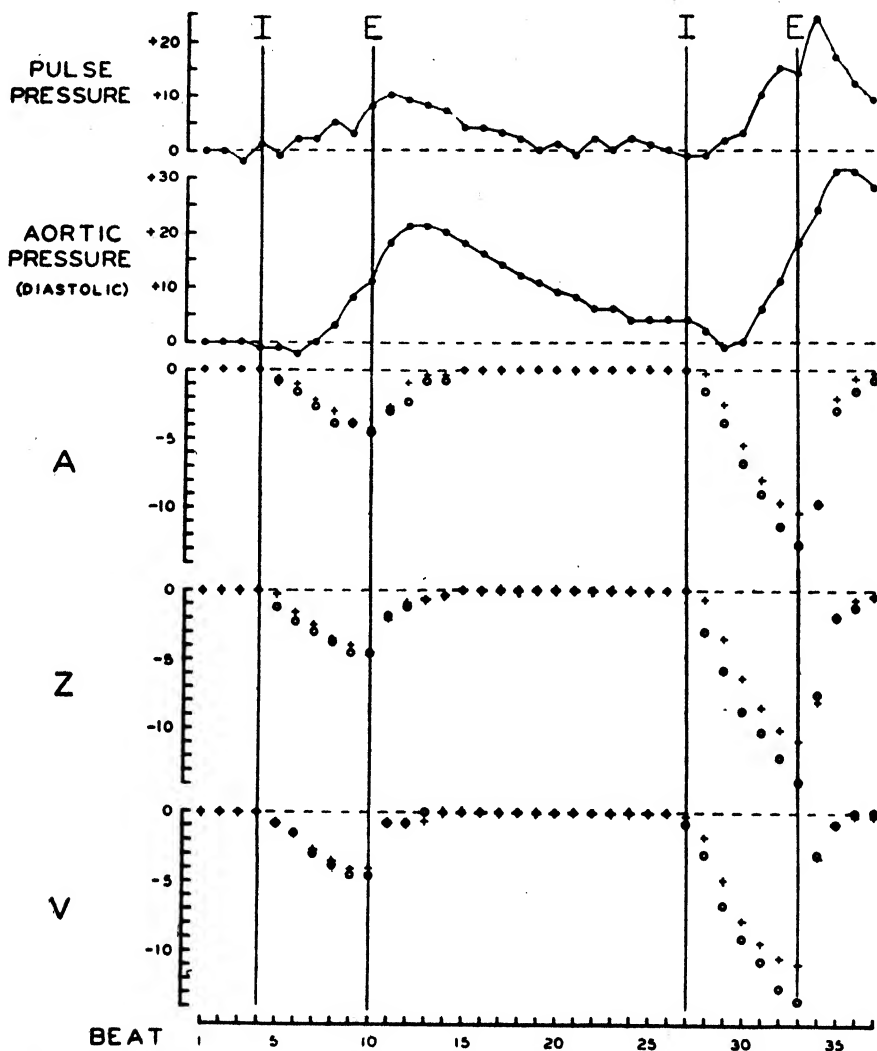
Fig. 3. RELATION of pulse pressure and aortic diastolic pressures to right atrial pressure and extracardiac pressure measured at corresponding A, Z and V points.   In lower three records: open circles = atrial and crosses = extracardiac pressures.   Changes expressed in mm. Hg from control expiratory beat.

Two respiratory cycles are shown; the first after vagotomy, the second with a mild tracheal occlusion during inspiration.   *I*, onset of inspiration; *E*, of expiration.

sure was slightly greater at the peak of inspiration after vagal section.   In four experiments a slight increase in effective pressure occurred during inspiration, but again this was of small magnitude (1 mm. Hg or less).   In only one instance following vagal

section was an increase of effective atrial pressure of 3.7 mm., 2.2 mm., and 3 mm. Hg noted at the *A*, *Z*, and *V* points, respectively.

Figure 3 shows a plot of the relative pressure changes within and around the right atrium as measured at the *A*, *Z*, and *V* points during a deep inspiration and expiration. The very close correspondence between changes in intra- and extra-atrial pressures is remarkable. During the second respiratory cycle plotted the tracheal tube was slightly occluded during inspiration only. This has the interesting effect of reducing intra-atrial somewhat more than extracardiac pressure, decreasing rather than increasing effective venous pressure.

TABLE 1. CHANGES IN AORTIC DIASTOLIC PRESSURES (EXPRESSED IN NUMERATORS) AND EXTRA-AORTIC PRESSURES (MINUS QUANTITIES IN DENOMINATORS) DURING CONTROL BEAT IN EXPIRATION AND IN CONSECUTIVE INSPIRATORY BEATS

EXPT.	EXPIRATION CONTROL BEAT	INSPIRATION BEAT NO.					
		1	2	3	4	5	6
1-4	150/−4.1	147/−5.8	147/−7.8	148/−7.9			
1-5	103/−4.8	102/−5.7	101/−9.8				
2-2	86/−13.5	76/−18.5	74/−23.				
2-9	116/−11.5	114/−11.2	109/−14.6	105/−16.2	108/−16.4	109/−16.6	
3-6	103/−6.9	101/−7.3	96/−8.7	89/−16.4	85/−14		
4-3	113/−3.9	112/−4.1	107/−4.8	106/−6.1	106/−7.0		
5-4	90/−6.4	88/−8.9	83/−11.1	86/−11.2	85/−11.2		
6-5	100/−4.6	91/−8.4		94/−9.1	93/−10.8	97/−11.4	97/−14.6
7-2	108/−6.9	107/−6.9	106/−7.9	105/−8.7			
7-4	118/−6.0	117/−6.9	117/−7.4	118/−8.6	119/−9.1	120/−9.1	129/−11.2
8-2	109/−5.8	108/−6.0	105/−7.1	102/−8.6	106/−8.8		
8-6	96/−5.9	94/−7.2	93/−8.4	99/−9.1	100/−9.8	107/−10.2	
9-6	88/−6.4	87/−6.9	86/−8.0	85/−9.1	86/−10.2	87/−11.0	
10-4	94/4.7	90/−5.8	89/−7.9	88/−8.0			

All figures in mm. Hg.

Many observations such as these have failed to show that the act of inspiration has any effect in drawing blood into the thorax or in altering the systolic discharge of the right ventricle.

Since the intrathoracic cannulae also overlay the aortic region it was possible to determine the degree of correspondence between changes in diastolic-aortic pressure and extra-aortic thoracic pressures at the same moment. Obviously this needed to be done in experiments in which the heart cycle was absolutely constant. This was possible in a number of barbital anesthetized dogs, but in most instances could only be accomplished by vagus section. In the simplest types of records, one of which is illustrated in figure 2A, systolic and diastolic pressures decrease during inspiration, the latter slightly less than the former, i.e., the pulse pressure decreases a little. Calculations of the three inspiratory beats revealed that the decline of diastolic pressure compared to a preceding normal beat equaled 4, 5 and 6 mm. Hg respectively. The corresponding extra-aortic pressures for the same three beats changed much less, being equivalent to 1.1, 3.2 and 3.3 mm. Hg. This relationship was rather con-

sistently found in all experiments during the initial portion of the inspiratory cycle (table I). Obviously the decline in diastolic aortic pressure is greater than the reduction in extra-aortic pressure and the latter cannot be used to give a quantitative estimate of the change in intrathoracic pressure around this vessel or the right atrium.

When inspiration becomes deeper and prolonged after vagal section quite a large number of beats occur during this cycle, as shown in figure 2C. In such a case the diastolic pressure decreases during the first two or three beats only; thereafter it rises and the pulse pressure becomes even greater than in control expiratory beats. A detailed plot of numerical values showing the magnitude of changes in diastolic pressure compared to corresponding alterations in aortic pressures is shown in the plot of figure 3. This plot also reveals that the augmentation of diastolic and pulse pressures is carried over into the phase of expiration and only slowly returns to control levels during the subsequent phase of respiratory apnea.

Since the configuration of aortic pressure pulses is not changed, such alterations in pulse pressure may safely be used as an index of directional changes in systolic discharge of the left ventricle. According to such a criterion it seems apparent that the systolic discharge is slightly decreased during the first two or three beats of an inspiratory act, but if this is prolonged the systolic discharge becomes progressively greater and exceeds the control.

<div align="center">DISCUSSION</div>

The results obtained by comparing instantaneous pressures in regions around the right atrium and aorta with pressures within these portions of the circulatory system are considered important from a number of angles.

Under normal conditions and an absolutely regular cardiac rhythm the inspiratory changes of instantaneous pressures in the right atrium measured at the summit of atrial systole (A), onset of ventricular contraction (Z), and beginning of ventricular filling (V) are identical with pressure changes in the surrounding intrathoracic space. However, this has been demonstrated only in dogs during natural respiration and under normal conditions; it must be applied with caution to man in abnormal conditions. On the contrary, the changes of intra-aortic pressure are by no means a reliable criterion of changes in pressure around the right atrium and aorta and may not be used in calculations of changes in effective venous pressure.

An analysis of changes in right and left ventricular stroke volumes during inspiration based solely on probable changes in effective right atrial pressures and changes in aortic pulse pressures leads to the following conclusion: When not more than three cardiac beats occur during inspiration, right ventricular filling and presumably systolic discharge remain unaffected; but the left side stroke volumes are reduced. This explains the reduction in pulse pressure and fall in diastolic pressure which is greater than the decline in extra-aortic pressure. This combination of dynamic events favors the view that blood is stored in the lungs during early inspiration.

When more than three heart beats occur during inspiration, aortic diastolic, systolic, and pulse pressures all increase, even considerably beyond apneic control

levels, as shown in figure 2C and D.   It is difficult to interpret these changes otherwise than that the systolic discharge during the fourth to sixth inspiratory beats are increased much more than the stroke volumes of the first three inspiratory beats were decreased.   The net effect is an increase in cardiac output during inspiration.   However, the effective right atrial pressure changes (fig. 3) offer no evidence that the output of the right ventricle is changed at all during prolonged deep inspiration.   One of two possible deductions can be drawn; either the pulmonary circuit yields an additional supply of blood to the left heart through mechanical effects of lung inflation *or* changes in effective right atrial pressures, even when meticulously measured, do not offer a trustworthy criterion of inspiratory changes in venous return which are sufficient to affect right ventricular filling and discharge and, after a delay, the left atrial filling and left ventricular discharge.   No new evidence was obtained as to which of these mechanical factors operates solely or dominantly.   However, recent studies (9) have shown that owing to the large capacity of the atrio-caval system small changes in venous return may not cause measurable differences in right atrial pressures although left atrial pressures are affected.   Since it is difficult to visualize any scheme of pulmonary mechanism by which blood is retained during the early phase and moved forward during a later phase of prolonged inspiration, it appears probable that alterations of venous return and right ventricular output are responsible, even though right atrial pressure changes are not detectable.

SUMMARY

1. A technique is described by which changes in right atrial and aortic pressures during inspiration and expiration can be compared with simultaneous changes in intrathoracic pressure immediately around these structures.

2. Calculations of effective right atrial pressure at three moments of the cardiac cycle—height of atrial contraction, beginning of ventricular systole and onset of ventricular filling—failed to reveal evidence that the act of inspiration augments the return of venous blood and right ventricular input.

3. However, this inference must be tempered by a realization that a fair increase in venous return may occur without a measurable increase in effective venous pressure (9).   That this occurs also during inspiration is strongly suggested by coincident registration of other dynamic changes.   When six or more heart beats occur during a long deep inspiration, such as follows vagotomy, aortic, diastolic, and pulse pressures increase after the third beat of an inspiration, while effective right atrial pressures remain unchanged.   It is difficult to conceive of a type of pulmonary mechanism whereby blood is apparently retained during the early phase of inspiration and larger quantities are moved toward the left side during late inspiration,—all without any change in stroke volumes of the right ventricle.

The conclusion follows that failure to detect measurable differences of effective right atrial pressure does not necessarily preclude the occurrence of changes in right ventricular filling and discharge during inspiration.

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CARDIODYNAMICS OF EXPERIMENTAL INTERVENTRIC-ULAR COMMUNICATIONS[1]

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NATURAL defects of the interventricular septum caused by developmental arrest are unquestionably accompanied by slow compensatory functional reactions on the part of the two ventricles. Their nature can only be inferred from autopsy studies. However, such interpretations may apply to end results caused by decompensations rather than to true dynamic consequences of the lesion *per se*.

For this reason the immediate dynamic alterations following an artificial communication between the ventricles of mature dogs was studied with the hope that the dynamic alterations may be applicable to defects which are produced naturally. A large external shunt rather than a surgical fenestration of the septum was used for two reasons: 1) surgical defects comparable in size to those that occur naturally are difficult to produce without involvement of the conduction system and consequent alteration of the dynamics of the heart beat; 2) immediate and sequential dynamic alterations can be better evaluated when a communication exists which can be alternately opened and closed.

METHODS

Dogs under morphine-barbital anesthesia, weighing from 10.5 to 20 kilograms, were subjected to the following surgical procedures: cannulation of femoral vein, trachea, carotid artery and midsternal exposure of the heart. The heart was cradled in the pericardium, care being taken not to embarrass venous return or heart action. Interrupted positive air pressure was used for artificial respiration.

Shunts of numerous designs and sizes were tested. The one finally found to be most suitable is shown in figure 1. It consisted of two brass cannulas of 7 mm. bore, the one for the left ventricle being slightly longer than the one for the right. These were connected by a short segment of heavy rubber tubing. A strong hemostat on the tubing served to open and close the communication.

Intraventricular pressures were recorded by calibrated manometers of the Gregg type. In early experiments, number 16-gauge needles connected by flexible lead tubing to the manometer were thrust through the walls of the ventricles at points on the heart which were observed to show the least movement during contraction and relaxation. In later experiments they were combined with the shunt, as shown in figure 1. Aortic pressure pulses were similarly recorded by a sound passed down the carotid artery. Heparin (0.4 cc/kilo of a 1 per cent solution initially, followed by 0.5 cc. every half hour) was used intravenously to prevent clotting.

After control pressures had been recorded simultaneously from both ventricles by hypodermic needles inserted through the ventricular wall, the interventricular shunt was inserted in the following manner: The needles were removed from the ventricular walls and long-jawed, thin scissors inserted

into the needle holes.   By opening the jaws, the superficial sinospiral fibers were separated until a hole large enough to admit one end of the external shunt was obtained.   One cannula completely filled with saline was first pushed into the left ventricle.   A hemostat clamped on the heavy rubber tubing kept blood from escaping from the left arm of the shunt, while the right cannula was being inserted. The two arms were then immediately joined by the rubber tubing and connections with manometers and needles were made.

The effects of two types of experimental maneuver were recorded.   In one, simultaneous right and left ventricular and aortic pressures were recorded and the shunt closed during the period of registration.   In the other the shunt was opened during the recording period.   Records of stabilized effects were taken one to two minutes later.   Pressure changes described below were calculated from the averages for the ten beats immediately before and after the opening or closing of the shunt. It was not possible to determine the changes in the first or second beats after the experimental maneuver because of artifacts and occasional extrasystoles induced by the manipulation of the hemostat. Each record thus consisted of three parts: 1) control, 2) the opening or closing of the shunt including at least the next 10 beats and 3) a record taken one to two minutes after the experimental maneuver. This procedure did not prove universally successful.   Of 26 experimental animals, 12 fibrillated during the insertion of the shunt.   One heart suddenly became hypodynamic, but did not fibrillate.   Of the remaining 13 successful experiments 4 were discarded because of the poor contours of the curves

RESULTS

The changes in pressure pulses from the left and right ventricles and the aorta after opening and closing the shunt are illustrated in figure 2 by one of the records from the nine good experiments.

Comparison of segments *A* and *B* reveal that opening of the shunt elevates initial intraventricular pressure (9<11 mm. Hg) and the maximal pressure (27<34 mm. Hg) in the right ventricle.   The initial pressure in the left ventricle also rises (4<7 mm. Hg), but the pressure maximum declines (77<64 mm. Hg).   The obvious reductions in systolic, diastolic, and pulse pressure in the aorta indicate that the systolic discharge of the left ventricle was reduced.   The heart rate slowed a little (154 >149/ min.).

In order to restore the stroke volume of the left ventricle and the aortic pressures approximately to normal levels, a continuous slow infusion of warm saline was given in this experiment.   The improvement which resulted while the shunt remained open is illustrated in segment *C*.   The shunt was then closed with the effects depicted in segment *D*.   Comparison of these two records reveals that closure of the shunt reduces the initial tension (12>8 mm. Hg) and the maximal pressure (34>29 mm. Hg) in the right ventricle, while it increases the pressure maximum in the left ventricle (81<98 mm. Hg), but causes no measurable change (8 mm. Hg) in the initial pressure. The heart rate increased slightly (151<153/min.).

A total of 46 records from nine good experiments similar to those of figure 2 was measured and the results tabulated.   This included data concerning changes in heart rate, initial and maximal pressures in the left and right ventricles during control states, immediately and some time after opening the shunt and immediately and after reclosing the shunt.   In addition, the duration of systole and its relation to cycle length (S/C ratios) were measured.   Changes in aortic pressure pulses were evaluated.

Initial and maximal ventricular pressures.   The immediate effects of opening and closing a shunt on right and left ventricular pressures are schematically summarized

by the diagrams of figure 3.   In 18 records *opening* the closed shunt caused the follow-
ing immediate effects: In the left ventricle maximal pressures decreased in all cases
from 2 to 24 mm. Hg; initial pressure fell in one record, remained the same in 7 and
increased in 10, the change varying from −1 to +3 mm. Hg.   In the right ventricle
maximal pressures increased in all cases from 2 to 17 mm. Hg; initial pressure fell in
no case, remained the same in 7 and increased in 11, the change varying from 0 to
+ 6 mm. Hg.

In 15 records *closing* the open shunt produced the following changes: In the left
ventricle maximal pressures increased in all cases by 5 to 20 mm. Hg; initial pressures
fell in four cases, remained the same in eight and increased in one, the change varying
from −1 to +1 mm. Hg.   In the right ventricle maximal pressures decreased in all
cases by 3 to 15 mm. Hg; initial pressure fell in 3 cases, remained unchanged in 7 and
increased in 3, the change varying from −2 to +1 mm. Hg.

Fig. 1. DIAGRAM illustrating the principle of an
interventricular shunt and the registration of intra-
ventricular pressures.
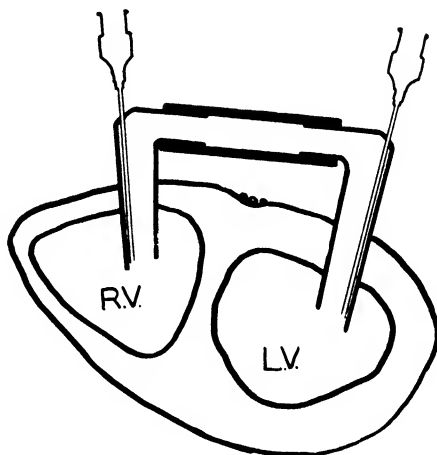
Heart rate and systole/cycle (S/C) ratios.   In half of the experiments opening and
closing of the shunt produced no changes in heart rate.   In the others a slight in-
crease occurred during opening and a slight decrease during closing of the shunt.
Since these changes needed to be taken into account in determining the effect of inter-
ventricular septal defects on the duration of right and left ventricular contraction,
the well known expedient of determining systole/cycle ratios (S/C ratios) was used.
Opening of the closed shunt generally caused the S/C ratio to increase in the left
ventricle; in other words, contraction was prolonged with respect to cycle length
(fig. 4, A—C).   Although the peak of systolic pressure came earlier in the cycle
(fig. 4, A—B), the incisura came somewhat later and the result was a greater duration
of systole.   While the increase in the S/C ratio was not found in every record, in the
majority of experiments the ratios indicated a prolongation of contraction by 5 to 10
per cent.   The changes in S/C ratios generally paralleled alterations in initial ten-
sion.   In these experiments closing the shunt caused the S/C ratio to shorten.
Opening the shunt caused a more variable change in the right ventricle.   Three ex-
periments had records which showed an abbreviation of the S/C ratios, although
other curves in the same experiments showed the expected increase (fig. 4).   Other

experiments analyzed showed the S/C ratio to increase consistently when the shunt was opened.

The onset of systole preceded in the left ventricle when the shunt was closed in six experiments (fig. 4). Precedence of the left varied within the same experiment
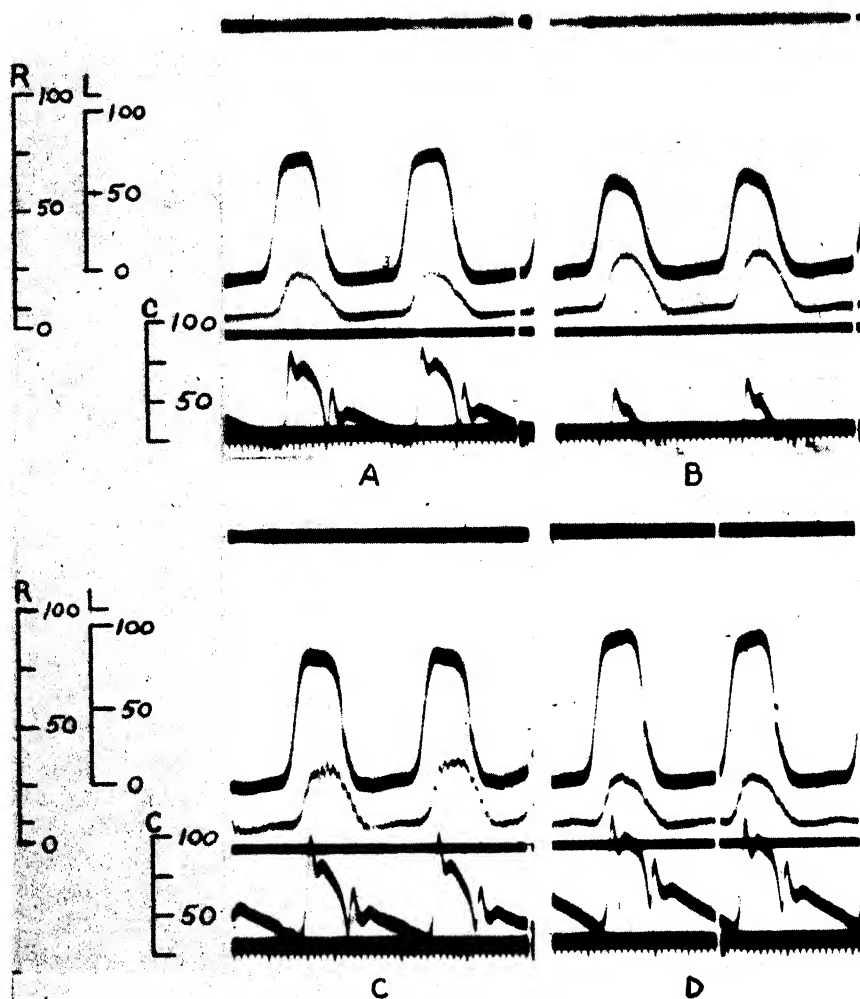
Fig. 2. *A*, PRESSURE PULSES from the left ventricle (upper), right ventricle (middle), and carotid artery (lower) under control conditions. *B*, effects of opening the shunt. *C*, effect of raising systolic discharge by venous infusion with shunt open. *D*, effect of closing shunt. Calibrations on right.

only slightly--not more than .01 second in any case. The time by which the left preceded the right in these six experiments varied from zero to .070 second. In most of the records from two experiments the ventricles contracted simultaneously. *Opening* the shunt did not alter the respective beginnings of systole in the ventricles appreciably (fig. 4). The chief importance of these observations is that they give assurance that the dynamic alterations of the two ventricles reported were not due to abnormalities of ventricular excitation.

Changes in contours of right and left ventricular pressure pulses.   As illustrated in figures 2 and 3, opening of the shunt caused the systolic pressure peak in the left ventricle to come earlier in the cycle.   From this peak the pressure declined considerably during the remainder of systole.   In other words, the pressure is not sustained owing to the decrease in total resistance occasioned by the shunt.   The decline of pressure during isometric relaxation also occurred more quickly in the left ventricle when the shunt was open (fig. 4, C—D).   Closing this shunt resulted in opposite effects. In the right ventricle opening the shunt caused no decided change in the peak of systolic pressure as indicated in figures 3 and 4 (A—B).   However, the summit usually
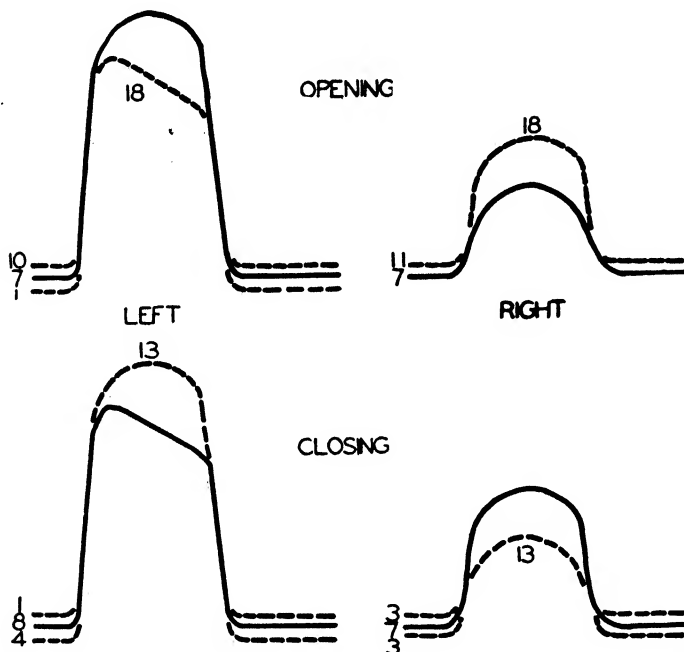
Fig. 3. DIAGRAM summarizing the effects of opening and closing shunt on left and right ventricular pressures.   Numerals indicate the number of cases in which the initial pressure changes indicated by dotted lines were found.

broadened.   The duration of isometric relaxation was sometimes increased and sometimes decreased (fig. 4, C—D).   When the shunt was closed the effects again were more variable on the right side.

Effective pressure gradient between left and right ventricles on opening the shunt. It is obvious that the pressure gradient available for shunting blood from the left to the right ventricle must vary during successive moments of systole.   The magnitude of these differences was determined by superimposing the right and left ventricular pressure curves brought to common ordinate values by means of Green's coordirectograph.   In figure 5, graph *A* shows the relation of the two pressures with the shunt closed.   The diagonally shaded area in *B* indicates the effective pressure differences which eventuated in an actual experiment with the shunt open.   As a result of a decline of pressures on the left and an elevation of pressures in the right

ventricle the overall effective pressure difference is much less than normal pressure relations without a shunt lead one to believe.   The rôle that a compensatory increase
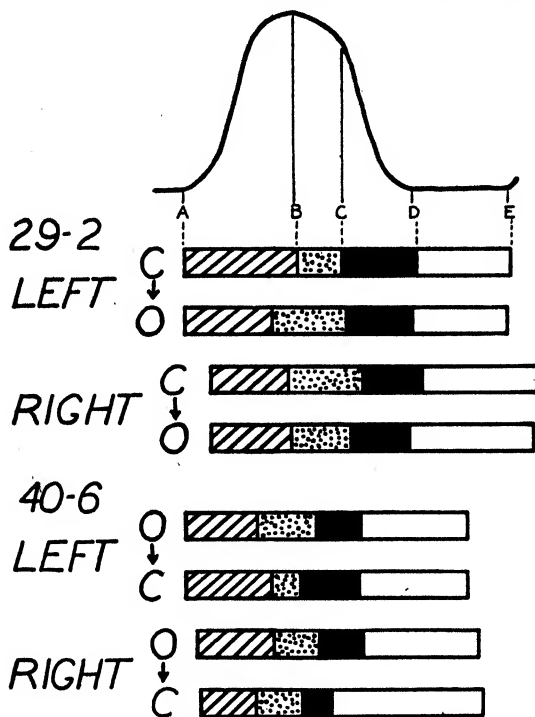
Fig. 4. DIAGRAM   illustrating changes in duration of different phases of contraction and relaxation and relative differences in the onset of right and left ventricular contractions. Two experiments are plotted.
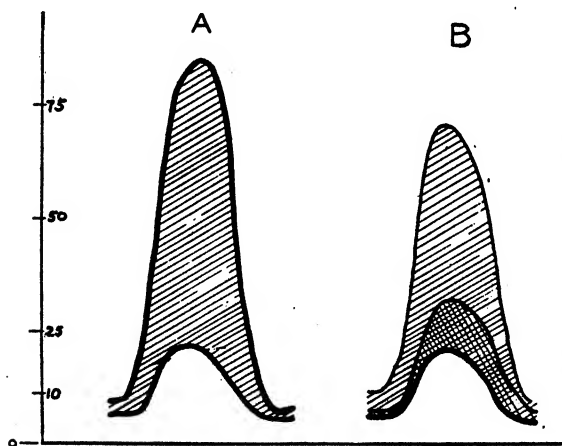
Fig. 5. GRAPHS   from   actual records indicating pressure differences between the left (upper) and right (lower) ventricular cavities. *A*, normal pressure differences; *B*, diagonally shaded area, pressure differences during opening of an interventricular shunt under experimental conditions. Cross-hatched area indicates extent to which physiological responses of right ventricle reduce the differential pressure between the two ventricles.

in right ventricular pressure plays in reducing the pressure differences can be graphically expressed by drawing also a curve of the normal right ventricular pressure.   In figure 5B the crossed hatched area represents the effects of right ventricular compensation.

INTERPRETATIONS

The data presented allow certain deductions as to the manner in which the mechanism of ventricular action is altered by the presence of an interventricular communication. Certain elementary physical facts are obvious. Since the pressure during systole reaches higher levels in the left than in the right ventricle, part of the stroke volume of the left ventricle must be diverted into the right ventricle while less is expelled into the aorta. As a consequence, aortic pressure declines and right ventricular pressure increases during systole.

One question appears never to have been adequately analyzed. Is the higher right ventricular pressure maximum solely due to a summation of pressure energy developed by the right ventricle with that transmitted from the left? Or does the right myocardium create more pressure energy during systole by virtue of certain dynamic changes? The latter may be expected to occur provided the right ventricle is dilated sufficiently to increase the initial length of its fibers. That such a stretch occurred in 11 or 18 experiments is obvious from the fact that initial tension was measurably increased. In 7 experiments however no such change occurred. This does not necessarily signify that no distension took place in the right ventricle during diastole. In a preceding paper (1) evidence was submitted that a considerable increase in volume of the large veins and right atrium can occur before a significant elevation of right atrial pressure supervenes. Similarly, at least in the exposed heart, careful observations and rapid motion pictures have shown (2) that the surface of the filled right ventricle below the origin of the pulmonary conus appears lax, suggesting that some available space exists which can be occupied by additional blood before the fibers are stretched sufficiently to cause a measurable increase in intraventricular tension. It is as though the heart were at first a plastic organ which dilates to accompany the increased volume of blood perhaps with only a minute and unmeasurable increase in initial tension. However, as filling increases more and more the elastic properties come into play with a greater increase in diastolic pressure. For this reason the measurable increase in initial tension found in 11 of our experiments is highly significant and justifies the conclusion that the excess pressure energy developed in the right ventricle is in part at least occasioned by a greater myocardial response.

This compensatory response of the right ventricle serves two useful purposes: 1) It enables the right ventricle to discharge into the pulmonary artery a total volume equal to that which enters from the right atrium during diastole and from the left ventricle during systole. 2) By more nearly equalizing pressures to the right and left of the septum the shunted volume of blood is less than that which would otherwise be transferred according to physical laws. In other words, the magnitude of the shunted volumes depends on the capacity of the right heart to compensate through increase in initial length and tension. Owing to the drastic procedures required in our experiments it is improbable that the compensation was as great as may be anticipated in human developmental defects. Indeed, when the shunt was left open for a long time with the hope that compensatory recovery of arterial pressures would supervene, it was sometimes found that the right ventricle distended more and more while arterial pressures and pulse pressures declined. As a rule a circulatory balance

was slowly restored after closing the shunt. In some of our hearts, however, the compensatory power of the right ventricle had certainly not reached its limit. This is shown by increased response of both ventricles to saline infusion, illustrated in figure 2, B and C. Indeed, this was found to be the most effective method for increasing the effective discharge of blood into the aorta, as judged by restoration of control pulse pressures.

In a condition in which the circulation becomes unbalanced by accumulation of a larger proportion of the total blood volume on the venous side an increase in total circulating volume is apparently an effective mechanism for restoring circulatory balance as long as the right ventricle can respond to the larger input load. The observation that right ventricular volume increased and that this frequently gave rise to an elevation of initial tension supports the theory which holds that hypertrophy so commonly found in human cases is a consequence of the increased distension.

The question may be raised why if the left ventricle empties—perhaps even more effectively than normally—through an opening in the ventricular wall as well as the aorta, the initial pressure should rise on the left side, and perhaps offer a stimulus for the development of hypertrophy on this side as well? The answer is apparently found in the fact that the right ventricle through development of a greater pressure maximum ejects larger stroke volumes into a pulmonary circuit whose resistance has probably not changed materially. This increased output of the right heart is reflected in a greater flow to the left.

The possibility must be considered that under certain conditions the left ventricle in ejecting blood through an interventricular septum does not largely do so at the expense of the blood volume normally expelled into the aorta, but utilizes part of its residual volume. This would explain the failure of initial tension to rise in the left ventricle in 7 of our experiments. Such a postulate must be thrown out of court, however, by the fact that the aortic pulse pressure was immediately reduced and gives evidence of a smaller systolic discharge. The failure of left ventricular initial pressure to rise may possibly be explained by the fact that the walls of the left ventricle are also capable of dilating in a plastic manner before initial pressure is significantly increased.

The normal myocardium adapts itself for the expulsion of large blood volumes by prolonging systole whenever initial pressure or diastolic stretch are increased (3). The increased S/C ratios found in most of our records on opening the shunt gives additional evidence that the natural response of the heart to distention remains unaltered in interventricular septal defects.

SUMMARY

The manner in which the heart reacts to an interventricular communication was studied in dogs by use of an external shunt which could be opened and closed alternately. The shunt and its application are described. The constancy of relations between onset of right and left ventricular systole gave evidence that the sequence of ventricular excitation was not disturbed by the apparatus. The dynamic changes, some of a compensatory nature, were deduced from detailed analysis of simultaneously recorded left and right intraventricular pressure curves aided by studies of

aortic pressure pulses.   Registrations were made by calibrated optical manometers of adequate efficiency.

The predominant changes in the left ventricle on opening a shunt consisted of an elevation of initial pressure, a decline in maximal pressure and a prolongation of contraction in relation to cycle length, with modification in the contour of pressure pulses.   The predominant effects in the right ventricle on opening the shunt consisted in an elevation of initial and maximal pressures.

An analysis of results lead to the conclusion that the great elevation of right ventricular pressure is certainly not caused wholly by summation of right and transmitted left ventricular pressures.   It is due in part to a more vigorous response of the right myocardium consequent to increased diastolic distension.   This compensatory response acts *a*) to mobilize larger volumes of blood for return to the left heart and *b*) to reduce the fraction of total left ventricular discharge which is transferred to the right ventricle during systole.

When the right ventricle fails to respond to the law of initial length and tension, progressive dilation and rapid circulatory failure supervene.   Increase in total circulating volume operates as one of the best compensatory mechanisms through additional right ventricular compensation provided the right ventricle is in good condition.

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COMPARISON OF CARDIAC OUTPUT BY A DIRECT METHOD AND THE HAMILTON-REMINGTON PROCEDURE

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D URING the course of concurrent cardiodynamic studies it became necessary to evaluate changes in stroke volume and cardiac output within short periods of time. On the basis of theoretical formulations and a few comparisons with other methods for determining cardiac output, it seemed probable that the method of Hamilton and Remington (1, 2) might serve our purpose. Since the validity of their calculations has only been checked against other indirect methods, it seemed important to make such comparisons with a method which measures cardiac output of the left ventricle directly. This seemed especially important in view of the fact that some of the theoretical postulates involved are subject to argument and that their scheme of analysis may not allow sufficiently for changeable factors of the arterial system.

This communication deals with attempts to compare calculated cardiac output by the Hamilton and Remington method with direct measurement of cardiac input.

METHODS FOR DIRECT AND INDIRECT MEASUREMENT OF CARDIAC OUTPUT

In order to calculate stroke volumes and cardiac output from aortic pressure curves, the latter were recorded in rather large amplitude by modified Gregg manometers. All pressure pulses used were those actually recorded at the same time that direct cardiac input determinations were made. Only records were used in which a stable diastolic pressure was maintained during the entire period. The curves were integrated by the procedures outlined by Hamilton and Remington (1, 2).

Since the validity of comparisons depends on the accuracy with which cardiac input is measured by our direct methods, these require detailed description. Three different procedures were used; or rather data from one procedure already available were used to avoid repetition, and two new techniques were developed.

1. Comparisons of measured venous inflow into the right heart and calculated left ventricular output. Four such experiments in which right ventricular input was estimated were available from records obtained by Wiggers, Guevara Rojas *et al.* (3). In these experiments the azygos vein was ligated and blood from the cannulated peripheral ends of the vena cavae was collected in a low level reservoir and pumped to a higher level reservoir from which it flowed through a Ludwig type stromuhr into the

right atrium. It is evident that this method does not measure coronary venous flow and that the actual input is thus underestimated, but certainly not by more than 20 per cent.
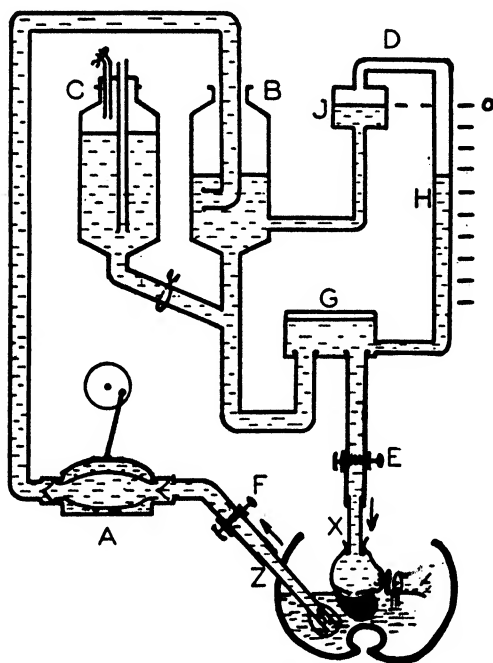
Fig. 1
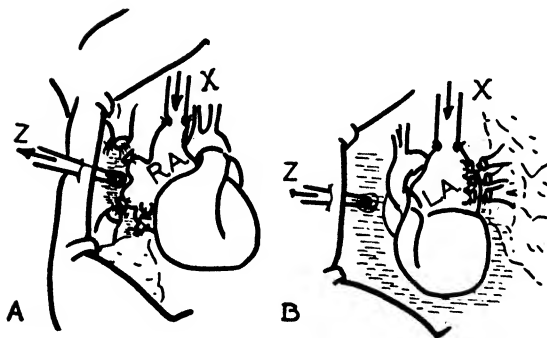
Fig. 2

2. *Comparisons of measured right cardiac input including coronary sinus flow and calculated left ventricular output.* In order to reduce the error, a method for perfusing the right heart was devised by which the coronary sinus flow was included in the measured right ventricular input. It consisted in principle in allowing blood from ligated and severed ends of the inferior and superior cavae *and* the coronary sinus

to gather in the thoracic cavity. A tube with a tip protected by a wire basket was inserted through an intercostal space so that it rested at the lowest point of the thoracic cavity. Through this tube blood was pumped into the right auricle via a large cannula inserted into its appendage. The apparatus is shown schematically in figure 1. A pump A with a small internal capacity and rapid strokes elevated blood to the chamber B whence it flowed into an inverted flask G which captured bubbles. From this chamber it passed by gravity into the right atrium. The input rate could be controlled by a screw clamp E. The rate of auricular inflow is determined by the device D which is an adaptation of the venturimeter principle. The difference of manometric levels corresponding to two points of the circuit (taken conveniently from B and G) can be read in tube H because the ampule J maintains the other level constant. The apparatus was calibrated after each experiment. In order to check inflow rates, complementary flow rate determinations were made by opening the Mariotte bottle C for a given interval of time while bottle B was temporarily closed. Aortic pressure curves were taken during the measured inflow period.

A few minor but important details of operation may be indicated. The entire attention of one experimenter is required to keep the circulation in operation. Cardiac input can be graduated at will by stopcock E as long as the capacity of the ventricle is not exceeded. Precaution must be taken to keep the tip of the thoracic cannula, Z, below the level of blood in the thorax in order to avoid the formation of foam in B. This is most conveniently done by graduating the degree of aspiration of blood by clamp F. Successful adjustment of clamps E and F results in a constant level in bottle B. The drainage of blood from the veins and the return to the right auricle are illustrated in figure 2A. The detailed steps of the procedure are as follows:

1. The chest is opened and an aortic optical manometer connected.
2. The two upper lobes of the right lung are ablated and the blood drained into the thorax containing heparin solution.
3. The azygos vein is tied and loose ligatures are placed around the two cavae and coronary sinus.
4. A tube with a wire protection at the end is inserted through an intercostal space, and its tip placed at the bottom of the thoracic cavity.
5. The animal is heparinized.
6. A large cannula is inserted into the right auricular appendage.
7. The circuit inside of the animal is filled with heparinized blood from a large donor dog and connected to the auricular and thoracic cannulae.
8. Sufficient heparinized blood is placed in the thorax to cover the thoracic cannula safely.
9. The coronary sinus is tied and the vessel slit allowing blood to flow into the thoracic cavity. The superior and inferior vena cavae are ligated and cut, allowing blood to flow into the chest.
10. The pump is started by another operator, which thereafter continues to supply the heart with blood from the external circuit.

3. *Comparison of measured left cardiac input and calculated output.* This procedure differed from the foregoing in that the left pulmonary veins were tied centrally and cut peripherally, allowing blood after passing the lungs to enter the thoracic cavity. From this it was pumped as before via a left auricular cannula directly into

the left heart, as illustrated in figure 2B. The steps are the same except for the following:

Step 2. Loose ligatures are placed around the 4 pulmonary veins of the left side.

Step 3. The whole right lung is ablated.

Step 6. The left auricular appendage is cannulated.

Step 9. As nearly simultaneously as possible the left pulmonary veins are tied and severed, allowing blood to enter the thorax.

The principle difficulty encountered when infusing into the left heart was the occurrence of coronary insufficiency due to air emboli. Minute bubbles which were not removed from the blood in *G* found their way to the coronary arteries and caused the heart to fail in 14 of 17 experiments. The temperature of the blood in the thorax averaged 32°C.

RESULTS

Comparison of measured right ventricular input and estimated output of the left ventricle was made in 15 records selected from four experiments available from previous work in this department. In these the venous inflow into the right heart minus the coronary flow was measured. In 50 records from four experiments the right cardiac input *including the coronary sinus flow* was compared with calculated left ventricular output. In 16 records from three experiments, comparisons of input into the left heart were made with the estimated output from the left heart.

The individual data of these experiments are incorporated in table 1. Changes in cardiac output in the same animal were usually achieved by altering venous inflow except in the observations followed by an asterisk; in these small doses of neosynephrine were administered. Figure 3 shows in much reduced size 17 of the 81 valid records calculated. The upper 11 records demarcated by a line were from experiments in which the total right ventricular input was measured directly and the lower 6 records exemplify experiments in which left ventricular input was measured directly. On each record are inscribed the calibration, the surface area of the dog (S.A.), the calculated minute output per dog in liters (C.V.), and the directly measured input in liters (M.V.).

A glance at these records shows that while the form of most of the pressure pulses is reasonably good the diastolic pressure was rather low and the pulse pressure large in most animals owing to the artificial conditions of the experiments. However, in six records (20-6; 21-11; 7-13; 7-14; 8-4) the diastolic pressure ranged between 60 mm. and 80 mm. Hg. In all of these instances, except experiment 8-4, the calculated minute output proved to be of the order of about twice the measured ventricular input.

A perusal of the data charted in table 1 indicates that even greater disproportions can occur. This is particularly pronounced in experiments 6 and 7 in which the calculated output of the left ventricle was sometimes four-fold greater than the measured input into the left ventricle. The data also show that while commonly the calculated values show directional trends in output determinations this is by no means always the case when cardiac input is increased or decreased purposely by raising the pressure head for inflow.

TABLE I

RECORD	CALC. OUTPUT, LT. HEART	RT. HEART INPUT, MINUS CORONARY FLOW
	cc./min.	cc./min.
88-2	1470	1056
88-38-39	1260	903
91-10	1480	894
91-11	1105	754
91-16	1100	540
91-20	1760	936
92-3	2020	984
92-7	1710	1280
92-8	1510	725
92-25	1970	1035
100-6	1340	1091
100-10	1550	672
100-20	1760	299
100-37	2294	812
100-42	3274	921
		TOTAL LT. HEART INPUT
		cc./min.
6-7	1008	490
7-4	1550	370
7-5	1630	400
7-6	1980	430
7-8	1890	475
7-9	2310	505
7-10	2350	505
7-11*	2300	485
7-12	1960	690
7-13*	1870	685
7-14*	2230	675
8-1	2290	1090
8-2	2690	1570
8-3	1560	1380
8-4	1140	1325
8-5	1670	1400

RECORD	CALC. OUTPUT, LT. HEART	RT. HEART INPUT, + CORONARY SINUS FLOW
	cc./min.	cc./min.
19-1A	1643	1875
3D	1644	1325
4C	2085	1690
5B	2320	1395
6A	2157	1235
B	1300	790
C	2385	1130
D	2514	1265
E	2065	1290
7A	2030	1290
8A	2295	1410
20-1	2370	995
2	2860	1255
3	3420	1155
4	2830	1200
5	2370	817
6	2220	1015
8	2030	860
9	1900	725
10	1780	560
21-1	1710	410
2*	1445	1285
3	2300	1360
4	1310	1185
5	2010	1325
6	1250	1290
7	2360	690
8	2190	1320
9	2350	1245
10	2540	1355
11	2520	1420
12	2860	1425
13	2880	1320
14	2840	1395
15	3280	1375
17	2540	1150
18	3440	1320
19	3220	1345
20	3290	1380
21	3580	1450
22	3290	1420
24	3700	1965
25	3760	1920
27	3660	2055
28	3560	1955
22-1	1505	770
2	1582	870
3*	1990	885
4	2185	927
5	2110	850

DISCUSSION

The astounding differences between measured blood volumes which enter the heart and leave it according to calculated pulse curve measurements clearly indicate
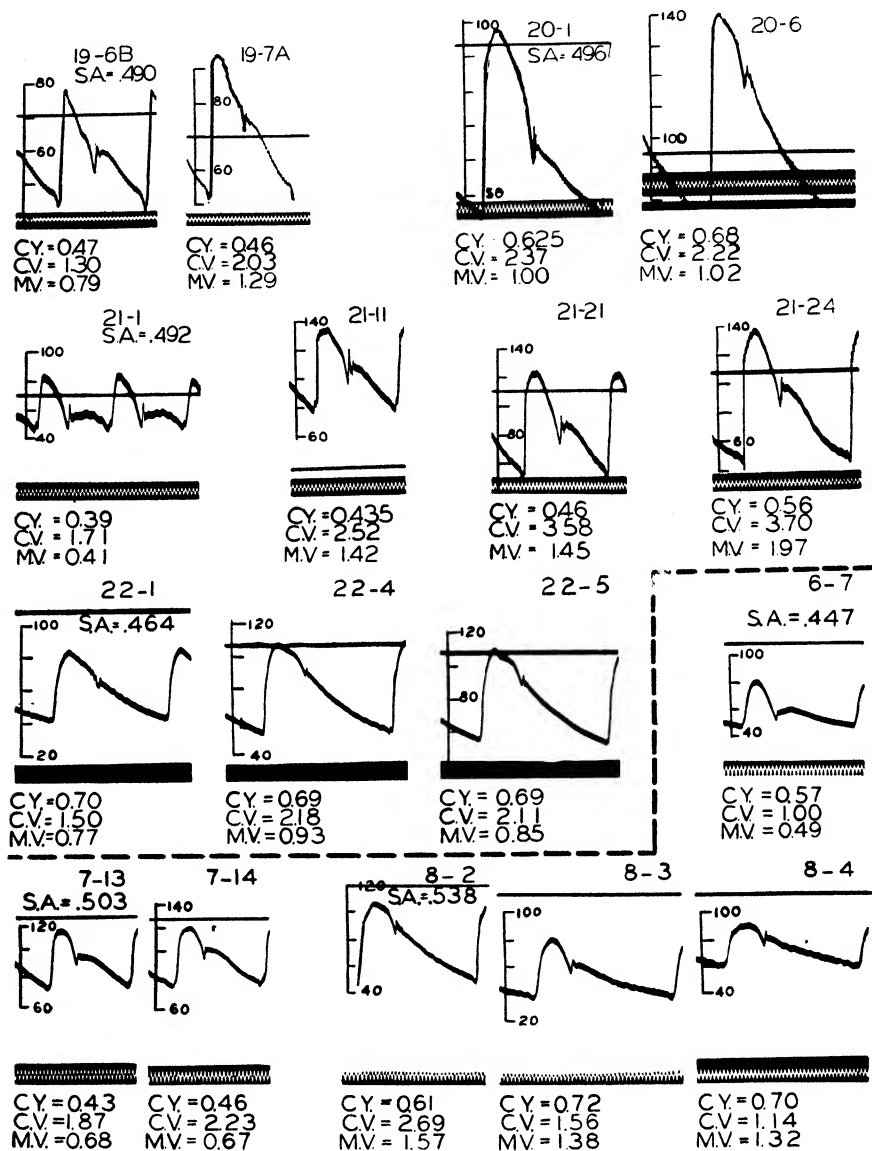
Fig. 3

that the Hamilton-Remington procedure for measuring cardiac output is far from good enough to be used in experimental studies. Some reservations should perhaps be exercised in concluding that equally large discrepancies exist in intact animals

between actual input and output calculated by the Hamilton-Remington method. They strongly suggest, however, that physical principles deduced from determination of averaged constants from dead dogs may not be generally applicable to individual live dogs, and possibly that uncontrollable factors may enter which invalidate their general employment.

It may be asserted with some firmness that discrepancies found in our experiments cannot be waived aside by insinuations that comparisons were made on failing hearts of moribund dogs and that they are therefore inapplicable to the intact circulation. If the postulates advanced by Hamilton and Remington are correct it is only necessary that the left heart eject blood in a reasonably normal manner into an arterial system with normal and unchangeable volume elasticity constants. Indeed, were it not for the coronary system an artificial pump operating in the same manner as the left ventricle could be substituted. No satisfactory artificial pump has so far been designed. The possibility exists of course that the arterial system changed in our experiments, but it seems highly improbable that they deviated from the normal more than in dead dogs from which Hamilton and his associates gathered the data used for generalized calculations for living animals.

SUMMARY

Three methods were devised by which the input of the right or left heart per minute could be measured directly, while aortic pressure pulses were recorded in large amplitude by calibrated Gregg manometers. The pressure pulses were analyzed according to the procedure of Hamilton and Remington and cardiac output was calculated.

The analysis of 81 records from 11 dogs revealed that left ventricular output is generally two or more times as large as the measured input; further that in isolated instances the directional trend of cardiac output was not even predictable from such analyses.

While some reservation must be exercised in stating that discrepancies of similar magnitude exist in less extensively operated dogs, the results clearly show that the method is far from good enough to be of use in experimental studies of cardiac output in dogs.

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EXPERIMENTAL HEMORRHAGIC SHOCK; A STUDY OF ITS PRODUCTION AND TREATMENT

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THE advances made in the study of shock have not resolved, in conclusive fashion, methods for the consistent experimental production of so-called irreversible hemorrhagic shock (1). The purpose of this investigation was to study the reasons for the inconsistencies which persist and to establish criteria which might lead to better definition of the degrees of shock.

Numerous methods for producing hemorrhagic shock have been described in recent years by Wiggers and Werle (2, 3), Wiggers, Ingraham and Dille (4, 5), Kohlstaedt and Page (6, 7), Glasser and Page (18), Seligman, Frank and Fine (8, 9), Walcott (10), Pasqualini (11), Sayers, Sayers and Long (12).

The principle of the procedure adopted in most of our experiments was described by C. J. Wiggers and Werle in 1942 (2, 3). Under general anesthesia, a dog is bled rapidly from a femoral artery into a reservoir containing heparin solution, until mean arterial pressure is indicated as 50 mm. Hg by a mercury manometer. This *moderate* hypotension is sustained for 90 minutes and is followed by a period of *drastic* hypotension at a mean arterial pressure of 30 mm. Hg lasting 45 minutes so that the total hypotensive period is 135 minutes. In their procedure, all the blood is then reinfused intravenously.

In our experiments two modifications of this technic were introduced. One is the maintenance of a permanently open connection between animal and recording reservoir under the desired positive pressure throughout the experiment. The other involves entirely replacing intravenous by intra-arterial transfusion of the removed blood (6, 7, 13, 14).

Cardiac intra-arterial transfusion has elicited little interest. The principle of the method was described as early as 1875 by Landois (15). In crude form, it was used successfully in an occasional patient by Halsted (16) in 1883 and by a few other surgeons (Huetner, Crile). A number of difficulties occurred subsequently and the method fell into disuse. Its revival was suggested to us by Colonel Sam Seeley, M.C., U. S. Army.

METHOD OF PRODUCING HEMORRHAGIC SHOCK

Dogs, weighing from 4.5 to 20.7 kg. and averaging 10.7 kg., were fasted for 24 hours and then anesthetized by subcutaneous injection of 5 mg/kg. of morphine sulphate and intraperitoneal injection of 30 mg/kg. sodium pentobarbital.[1]

A glass cannula (*A*, fig. 1) was inserted into a femoral artery, and connected through one branch of rubber tubing with a mercury manometer (*B*) recording arterial pressure on a slow-moving kymograph. The other branch lead to a recording

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[1] Several experiments carried out under local anesthesia (2% solution of novocain) produced results which were similar to those obtained with general anesthesia. In some of these experiments, sudden movements could not be avoided, and curare (intocostrin, 20 ʊ per ml.) was given intravenously in doses of 0.2 ml. at intervals of 5 minutes up to a total of 1.6 ml. Administration of curare in combination with severe hemorrhage led rapidly to serious respiratory difficulties and was therefore abandoned.

reservoir (C) in which the withdrawn blood was stored. A small side arm of this line was equipped with a stopcock adapter to which a Luer syringe could be attached for the purpose of injecting anticoagulant into the tubing.

The reservoir (C) was suspended on a balance spring and equipped with a pen to record changes in its weight on the kymograph. This allowed observation of the movements of blood back and forth from the animal to the reservoir during the course of the experiment. The stopper of the reservoir held two tubes; one short, through which the blood flowed and a long one through which air escaped or through which air could be pumped by a small bulb (D) to control blood movement between reservoir and animal. Pressure within the reservoir could be read on a mercury manometer (E) or a sphygmomanometer (F). An air outlet with adjustable valve
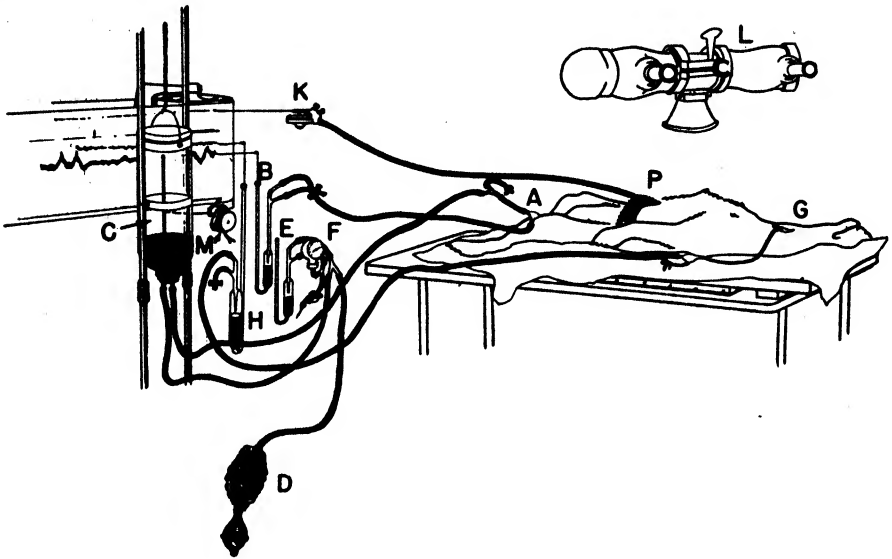
Fig. 1. APPARATUS FOR BLEEDING and arterial transfusion (see text)

was inserted in this line near the sphygmomanometer. This and the bulb (D) permitted complete control of air pressure in the reservoir.

A no. 12 French rubber catheter was inserted into the external jugular vein (G) with the tip approximately at the level of the atrium, and connected through a glass cannula to a water manometer (H) to record venous pressure on the kymograph. Respiratory rate was recorded (K) from a pneumograph (P). Time intervals of one minute obtained from a synchronous clock motor (M) were recorded on the base line.

Response of arterial pressure to a dose of 0.1 to 0.2 ml. of a 1:10,000 adrenalin solution was regularly ascertained before bleeding during hypotension, after transfusion. Rectal temperature, room temperature, relative humidity and barometric pressure were recorded for each experiment. Electrocardiograms and electro-encephalograms, hematocrit and hemoglobin values before, during and after hypotension, and radiographic examination of heart were made in a number of experiments (x-ray tube L).

PROCEDURE

Before the beginning of the experiment, all connections from the cannula in the femoral artery and catheter in the jugular vein to manometers and reservoir were sterilized and filled with heparin-saline solution (100 mg. heparin per 1000 ml. 1% saline).

Bleeding was started by releasing the clamp which closed off the line between cannula (*A*) and bottle (*C*). With the air outlet open, the blood entered the reservoir. Bleeding was done rapidly, at an average rate of 100 ml/min. When the mean arterial pressure reached 50 mm. Hg, the air outlet was closed. By manipulation of bulb and air outlet, the pressure was held at 50 mm. Hg for 90 minutes. During the period in which the blood flows from animal to reservoir, danger of clotting exists; consequently, 5 ml. of heparin-saline solution was injected from time to time into the side arm near the cannula (*A*). While injecting this solution, both connections from cannula (*A*) to manometer (*B*) and bottle-reservoir (*C*) were clamped off. If heparin solution was to be injected into the reservoir (*C*) alone or if the blood in the reservoir was to be 'washed' or thoroughly mixed with this solution, the short connection between cannula (*A*) and fork with side arm was clamped off.

After the 90-minute period of *moderate* hypotension at 50 mm. Hg, the pressure inside the reservoir was lowered by opening the air outlet near the sphygmomanometer (*F*). Further bleeding took place although the amount of blood required to drop the arterial presssure to 30 mm. Hg was small, averaging only 7 per cent of the total amount removed. The period of *drastic* hypotension at 30 mm. Hg was maintained for at least 45 minutes, after which all or part of the blood stored was reinfused into the same femoral artery, by increasing the air pressure in the reservoir. The average rate of infusion was 130 ml/min. Infusion was usually stopped when the mean arterial blood pressure reached about 100 mm. Hg.

Early in the investigation, between July 1945 and June 1946, 31 animals were submitted to this procedure to determine its effect on survival. Experimental data are presented in table 1.

Since the same system of numbering the columns is used in all tables, the following describes a typical table.

Experiment number is given in Column I, the amount of blood removed in ml/100 gm. body weight in Column II, time of hypotension in minutes in Column III. Column IV shows relative intake of blood by the animal during the hypotensive period, whereby o signifies no intake, x slight, xx moderate, xxx considerable and xxxx large intakes. Intake into the animal during transfusion to reestablish arterial pressure at the level of 100 mm. Hg in per cent of total volume of removed blood is given in Column V; Column VI shows the pressor response to adrenalin after reinfusion in per cent of the response to the same dose before bleeding. Column VII gives the survival rate of animals after the shock procedure; permanent survival is indicated by 'yes', temporary survival by the number of hours up to 36 and death within one hour after transfusion by 'o'. In each column the animals are divided into two groups, *I* and *II*, according to prognostic criteria which will be discussed below.

The average duration of hypotension for the experiments in table 1 was 143 minutes and the average volume of blood removed to produce shock was 5.2 ml/100

TABLE 1.   SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED ONLY WITH INTRA-ARTERIAL TRANSFUSION (EARLY CONTROL EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
22		4.6		135		0		27				Yes
23		5.5		150		0		37				27
25		4.8		142		0		47				2
26		2.6		135		0		64				10
43	5.5		139		XXX		69				0	
71	5.7		156		XX		100				0	
72	4.4		156		XX		100		80		Yes	
84		6.1		150		0		20		85		10
85	5.5		135		XXX		100				0	
100	5.2		135		XXXX		100				0	
102		4.7		150		x		39		100		Yes
103	4.9		135		XXXX		100				0	
105	6.0		141		XX		63		80		10	
106		4.6		144		x		29		100		Yes
107	4.1		135		XX		50				0	
108		3.5		136		x		60		80		Yes
111		4.2		150		x		31		66		10
112		6.1		137		x		33		100		5
113		4.4		152		x		31		40		14
115	6.5		141		x		79				3	
116	7.2		146		xx		79		30		3	
117		4.9		135		0		50				5
121		5.6		139		x		46				3
122	4.8		137		xx		50		50		5	
139	5.3		136		XXX		92				5	
140	5.2		135		xx		100		40		5	
141		7.8		159		x		42		40		10
142		5.6		158		0		100		75		Yes
143		5.2		147		0		50				10
167		5.9		139		x		40		50		Yes
187	4.3		136		XXXX		100				0	

Number of experiments in *Groups I* and *II*:

	Group I: 14	Group II: 17	Total: 31
Survival, permanent:	1 (7%)	6 (35%)	7 (23%)
Survival, 1 to 36 hours:	6 (43%)	11 (65%)	17 (54%)
Survival, less than 1 hour:	7 (50%)	0	7 (23%)

gm. body weight.   Of the 31 animals, 23 per cent survived permanently after transfusion and 54 per cent died within one to 36 hours (average 8 hours) while 23 per cent died within less than one hour.

The early series of 31 control animals was followed by a later series of 29 control

animals which were submitted to the shock procedure between September 1946 and January 1948. The experimental data for this group are presented in table 2.

Average duration of hypotension for these animals was 150 minutes and average

TABLE 2. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED ONLY WITH INTRA-ARTERIAL TRANSFUSION (LATE CONTROL EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL. ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
228		2.7		141		x		100				Yes
229		5.4		135		x		100		91		10
373	4.6		137		xxx		100		55		Yes	
375		4.8		137		o		50		81		Yes
376		3.7		135		o		40		100		Yes
377	3.6		141		xx		100		23		4	
378		6.6		141		o		46		100		Yes
381		6.3		142		o		89		86		Yes
382		5.6		137		o		55		53		Yes
383		4.1		151		x		67		100		Yes
384		4.1		145		x		67		33		Yes
385		5.0		167		o		67		37		Yes
386		5.3		150		o		53		65		Yes
387		5.3		157		o		57		35		Yes
388		4.3		153		o		75		56		Yes
389	4.6		143		xx		33		31		6	
390		6.1		159		o		56		100		Yes
391		8.2		154		o		88		75		Yes
392		4.3		142		o		66				Yes
395		4.9		156		xx		62		85		Yes
397		5.1		142		o		65		100		Yes
415	3.7		142		xx		100		30		5	
416		4.7		142		x		84		80		15
417		4.5		227		x		100		60		5
421		4.6		142		x		78		134		Yes
422		4.8		165		o		82		83		Yes
434		4.5		163		x		43		43		6
435		4.0		147		x		70		50		Yes
436		5.5		150		xx		56		36		Yes

Number of experiments in *Groups I* and *II:*

	Group I: 4	*Group II:* 25	Total: 29
Survival, permanent:	1 (25%)	21 (84%)	22 (76%)
Survival, 1 to 36 hours:	3 (75%)	4 (16%)	7 (24%)
Survival, less than 1 hour:	o	o	o

amount of blood removed was 4.9 ml/100 gm. body weight. Of the 29 dogs, 76 per cent survived permanently and 24 per cent lived from 4 to 15 hours with an average of 7 hours. None of the animals lived less than 4 hours after transfusion.

Despite the fact that there was wide difference in individual animals in their

response to hemorrhage , nevertheless, they could be divided into two groups according to the following criteria: *a*) The persistent tendency during the hypotensive period for the arterial pressure to fall below the level set and its corollary the tendency to take up blood from the pressure reservoir to maintain the 30 mm. Hg level of blood pressure proved to be a sign indicating poor prognosis even though the immediate response to transfusion may be good.   *b*) The amount of blood required to reestablish normal arterial pressure when the blood was given by way of the artery gave some indication for prognosis; when the whole amount removed or more was
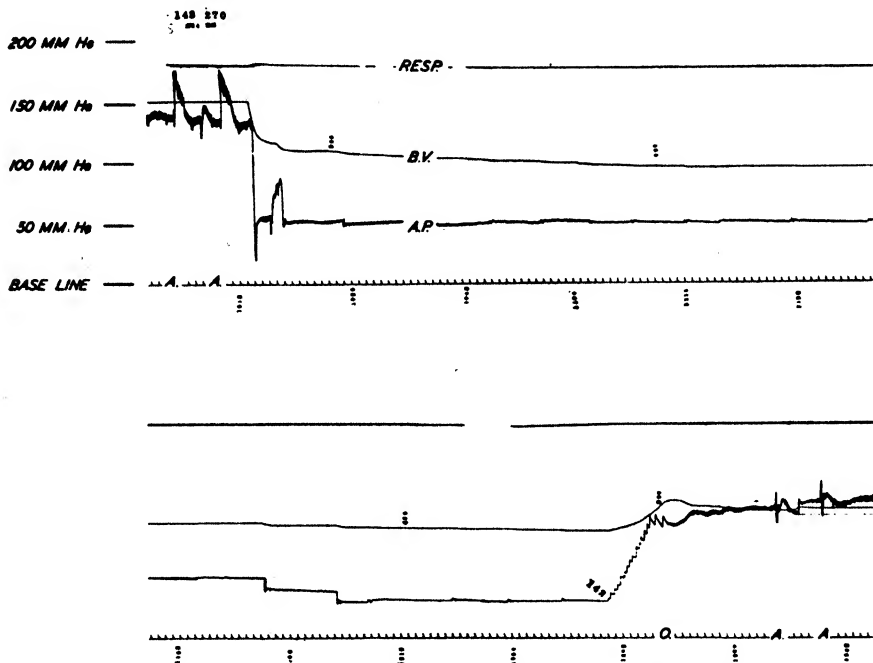
Fig. 2. RECORD OF DOG with good prognosis after hemorrhage.   Reading from above down 1) respiration (Resp.); 2) weight of blood in reservoir (B.V.); 3) arterial pressure (A.P.); 4) time in min. *A* signifies adrenalin injection.   *O* signifies ouabain injection.   Note lack of intake of blood during hypotensive period of 143 min., the small uptake to restore arterial pressure to control levels and the return of adrenalin responsiveness.

required, prognosis was poor.   *c*) When the pressor response to adrenalin after transfusion did not return to the prehemorrhagic control level, the outlook was not as favorable as when the response was as great or greater.

Thus dogs in which the prognosis was good showed no significant change in arterial pressure during the hypotensive period, a smaller intake of reinfused blood to restore normal arterial pressure (an average of 59 per cent of the blood removed as compared with 84 per cent in the animals with poor prognosis, given in tables 1 and 2, and finally, a greater relative responsiveness to adrenalin after transfusion (73 per cent of the control response as compared with 46 per cent in animals with poor prognosis).   As we have pointed out (19) none of these criteria alone is a re-

liable index to prognosis but taken together they yield reasonably accurate predictions.

We shall call *Group I* those animals in which, according to these criteria, the prognosis is bad, and *Group II* those in which it is good. In the 31 early control animals of table 1 in which no other treatment than arterial transfusion was employed, 14 fell into *Group I* and 17 into *Group II*. Only 7 per cent of *Group I* survived indefinitely, 43 per cent lived an average of 4 hours and 50 per cent died shortly after transfusion. On the contrary, of *Group II*, those with the criteria of good prognosis, 35 per cent lived indefinitely, 65 per cent lived an average of 10 hours and
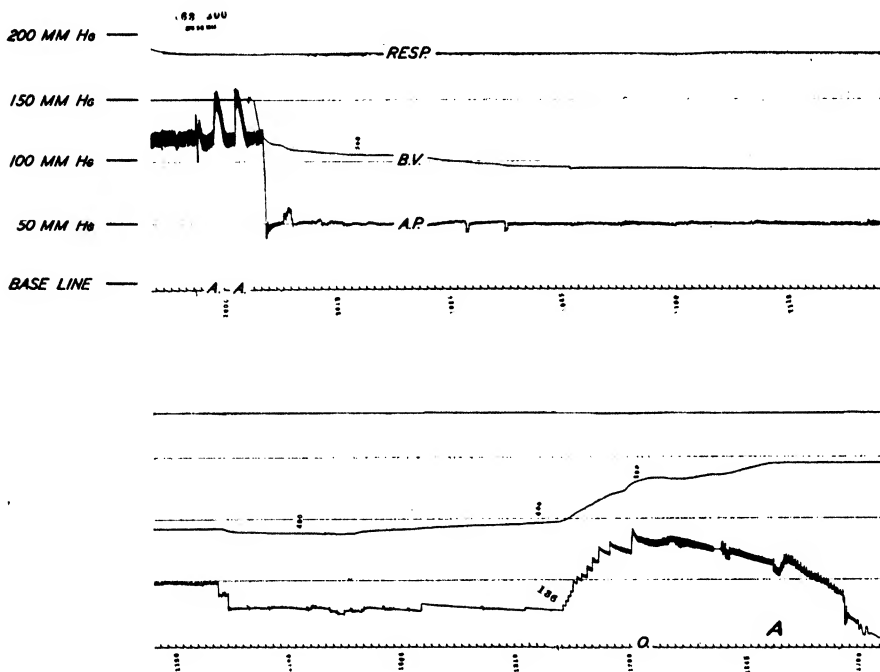
Fig. 3. RECORD OF DOG with bad prognosis after hemorrhage. Symbols as in fig. 2. Note intake of blood during the latter part of the hypotensive period, large intake to restore blood pressure to control level, poor response to adrenalin.

none died within less than 2 hours. Of the later series of control experiments (table 2) *Group I* comprised only 4 animals and *Group II*, 25 animals. One of the 4 animals in *Group I* and 21 (84 per cent) of the 25 animals in *Group II* survived permanently. The remainder lived from 4 to 10 hours and none died within less than 4 hours. Thus on the rigid basis of survival rates alone, the criteria indicate their usefulness.

Bleeding volume in all our shock experiments is given in terms of ml/100 gm. of body weight. The average amount of blood removed in all the experiments described in this paper was 5.4 ml. per 100 gm. body weight. Recalculation of blood removed on the basis of percentage of surface area instead of body weight did not yield a better correlation with the degree of shock or with survival rates. If the calculated bleeding

volume in percentage of body surface is taken as the basis for the amount of blood withdrawn, a relatively greater amount of blood was taken from large animals than from smaller ones. Using the formula $A = 11 \times W^{0.67}$, where A is the surface area in cm.^{2}, and W the body weight in gm., the surface areas for a 10-kg. dog and a 5-kg. dog are found to be 5265 cm.^{2} and 3309 cm.^{2}, respectively. Thus, the mean bleeding volume of 5.4 per cent of body weight means that 540 ml. of blood was withdrawn in the large dog and 270 ml. in the small dog. Thus 10.2 ml/100 cm.^{2} surface area were taken from the large dog and 8.2 ml/100 cm.^{2} from the small dog. Taking bleeding volumes per unit surface area as standard, our larger animals were therefore bled more severely than the smaller ones, although the difference of 25 per cent as indicated in the extreme example cannot be considered as decisive. Still the larger animals should have shown more severe shock and therefore lower survival rates. However, the percentage survivals for shock experiments are practically the same for both groups.

This was also borne out by an analysis of resuscitation experiments which will be presented later in this paper (table 6). Of 13 permanent survivors of that series the average weight was 9.4 kg. and the bleeding volume 6.0 ml/100 gm. body weight. For 26 nonsurvivors, the average weight was 10.2 kg. and the bleeding volume 5.8 ml/100 gm. body weight. The surface areas of the two groups are 5055 cm.^{2} and 5336 cm.^{2}, respectively, which makes the bleeding volumes in percentage of surface area 11.2 ml. and 11.1 ml/100 cm.^{2} surface area. This again indicates that there exists no definite correlation between survival and bleeding volumes measured either in percentage of body weight or body surface.

Although the averages of both bleeding volumes and duration of hypotension are rather uniform for the various groups of experimental animals, individual variations are considerable. While about 13 per cent of the animals used were unable to complete the prescribed period of 135 minutes of hypotension, there were several which withstood the hypotensive period for a much longer time. Thus, about 25 per cent of the animals which withstood more than 135 minutes of hypotension averaged 166 minutes with an extreme survivor of almost 4 hours. This extraordinary ability to withstand severe hypotension and still survive without obvious residual defects is also illustrated by one experiment in which after rapid bleeding the pressure was kept between 30 and 35 mm. Hg for 142 minutes. Following arterial transfusion the dog survived indefinitely, apparently uninjured. These observations agree with those of Kohlstaedt and Page (6, 7) in demonstrating the remarkable inherent ability of some animals to survive severe hemorrhage far more effectively than others. Thus to ascertain the effect of supposed therapeutic procedures, the investigator is faced with either using a larger number of animals, so securing a statistically valid result, or, alternatively, using objective criteria indicating the response of the body to hemorrhage. Both objectives were striven for in our studies. While the total number of dogs used in this investigation with all its ramifications was almost 500, we present in this paper only observations on 244 animals, all of which underwent a hypotensive period of at least 135 minutes.

Division of shocked animals into *Groups I* and *II* according to the prognostic signs described above proved of great value, since attempts to lump animals with

such manifestly different responses to the same experimental conditions into one group would easily produce misleading results.

We indicated several years ago that the response to adrenalin and angiotonin and the rate at which the heart dilated gave some indication of prognosis. In the past four years, measurement of the exchange of blood from a reservoir, in which there is a fixed pressure, with the arterial circulation has been added, along with the requirement of blood to refill the vascular tree at a normal load of arterial pressure. Essentially, these criteria seem to measure the ability of the animal to maintain a certain vascular capacity and to respond to changes in capacity which, in our opinion, may in a measure be reflected in the responsiveness of blood vessels to humoral stimuli. If this interpretation is correct, it is apparent that the inability to prevent arterial pressure from persistently falling below 30 mm. Hg during the hypotensive period indicates a tendency either to loss of peripheral resistance or to weakening of the heart, both of which occur in the terminal phase of shock. They occur early in some animals and it is these that we place in *Group I* as having bad prognosis.

While practically none of the animals with unfavorable prognosis survived the shock procedure, the fate of the dogs with favorable prognosis was not as clearly predictable. This is illustrated by a comparison of the survival rates for the early and the late control series as presented in tables 1 and 2. Although all of the experiments were performed by the same group of experimenters, there is a marked increase of permanent survivors in the control series in the later two years of the investigation, 84 per cent as compared to 35 per cent in the initial year of the work for *Group II* animals. Some factors which have contributed to this improvement are improvement in the skill with which the operations are carried out and better after-care of the animals. Blood clotting, which frequently interfered in the earliest experiments, was eliminated. Attempts to reinfuse quickly the *full* amount of blood which had been removed after the hypotensive period led in early experiments to irregular cardiac action and, when the overtransfusion was continued, occasionally to heart failure. The amount of blood reinfused was therefore reduced to an average of 70 per cent of that withdrawn. In addition to these obvious causes for increases in the survival rate in the course of our experiments, other obscure factors exerted possibly an even greater influence. This is demonstrated to some extent in the diagram, figure 4, which represents a correlation between permanent survival rate and date of experiment for 244 animals.

The dots in figure 4 represent the percentage of permanent survivals for two-week periods from June 1945 to April 1948. For the early period a continuous increase in the survival rates is evident. However, from July 1945 to the present there suddenly occurred periods extending from a week to several months during which prognostic criteria for most animals turned unfavorable. Such can be seen in figure 4 as low percentage average two-week survival rates around February 1946, April and May 1946, May 1947 and November 1947. The longest low survival period, however, extended practically over three months from February to May 1948 and occurred after experience had been gained on experiments with 440 animals. During this time there were several two-week intervals with no survivals. But results obtained on 5 shocked control animals during the week starting May 17, 1948 changed

this picture suddenly; all 5 control animals were given a good prognosis and they survived permanently. Various suggestions have been made to explain the sudden periods of low resistance to the shock procedure; these include illness of the animals, bacterial infection, seasonal effects, climatic conditions and sudden change of food and environment. We have been unable to correlate any of them with these changes.

It may be of interest to report here that the mean arterial pressure of 244 animals under pentobarbital anesthesia before bleeding was 130 mm. Hg with extremes of 70 and 185 mm. Hg. Mean arterial pressure one hour after transfusion was 98 mm. Hg with extremes of 60 and 150 mm. Hg for permanent survivors.

Although a variety of agents were tried during our experiments, only those which appeared to have a significant effect upon the shocked animal will be described. Ouabain was first used because Kohlstaedt and Page (7) had observed that cardiac dilatation after prolonged hypotension was an important sign of impending terminal shock and when it appeared treatment by blood transfusion alone failed. We have confirmed these findings and proceeded to investigate whether ouabain had beneficial
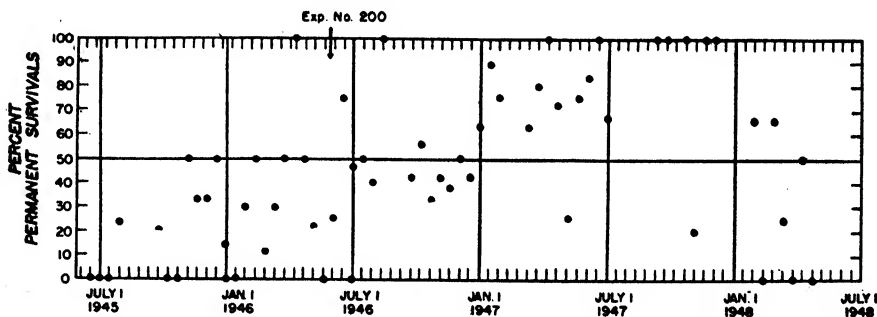
Fig. 4. Percentage permanent survival for 2-week periods from July 1945 to June 1948. No division is made between those with good or bad prognosis.

effects on the heart when given either during or shortly after arterial transfusion. Sixty-one animals were submitted to the shock procedure early in the course of our investigation (approximately throughout the same period of time as the control animals in table 1) and were given 0.05 mg/kg. body weight of ouabain intravenously during or shortly after the transfusion (table 3).

Twenty-two dogs had unfavorable and 39 favorable prognoses. Despite ouabain, all 22 of the former group died within 24 hours, the majority living 5 hours. In contrast 74 per cent of the 39 of the latter group survived indefinitely and 26 per cent lived an average of 7 hours. This contrasts with the control group in which 35 per cent survived while 65 per cent lived an average of 10 hours.

Later in the course of the experiments (table 4) 33 more animals were studied. Only 3 of these had poor prognosis and 30 good. Despite ouabain none of the former and 83 per cent of the latter survived permanently with 17 per cent surviving an average of 14 hours. This compares with 84 per cent survival and 16 per cent in the later control group.

Thus ouabain seemed to increase survival only in the early experiments in which prognosis was good. In this early group there was a tendency to overtransfuse by

TABLE 3. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION AND OUABAIN (EARLY OUABAIN EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
27		3.6		135		o		100				Yes
76	5.0		135		XXX		75		7		23	
77		6.0		180		x		60		93		Yes
78		5.6		172		o		32		100		Yes
79		5.6		152		x		52		46		Yes
80		4.3		153		x		34		19	3	
82		6.7		180		x		24		40	10	
83		4.4		180		o		36		100		Yes
86	4.8		150		XXX		80				1	
87		7.1		160		xx		50			10	
88	5.1		150		XXX		50		12		1	
89		5.0		145		x		19		88	10	
90	5.1		137		xx		39				10	
91		6.1		157		o		18		63		Yes
92	5.6		138		XXX		56		33		7	
93		5.0		153		x		16		100		Yes
94		5.8		167		o		36		70		Yes
95	5.5		137		XXXX		100			19	2	
97	5.2		143		XXX		43			30	2	
98	4.7		141		XXXX		36			'5	2	
99	5.8		142		o		55		50		5	
101		4.4		137		x		25		57		Yes
118		7.8		137		x		32		50		Yes
119		5.0		140		x		36		85	7	
123	5.2		135		XXX		83		60		7	
124	5.0		137		xx		62				o	
126		4.4		137		x		100		100		Yes
131	6.8		137		xx		50				3	
132		5.6		137		o		55		80		Yes
133		5.8		144		x		76		80		Yes
134		5.8		147		o		50		40		Yes
135		6.6		147		o		28				Yes
136	5.6		142		xx		53		50		6	
138		6.0		152		x		54		40		Yes
144		4.9		164		x		47		40		Yes
146		5.6		143		o		27		40		Yes
147		7.1		136		XXX		47		40		Yes
148		6.0		143		o		40		45		Yes
149		6.3		144		o		29		50		Yes
150	5.9		143		XXX		65				1	
154		4.4		149		o		37		65		Yes
155		4.2		135		o		73		100		Yes
156		6.0		163		x		42		100	6	
157		6.4		139		x		50		60		Yes
158		7.3		147		xx		33		20	6	
159		6.8		183		o		27		35		Yes

TABLE 3.—*Continued*

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
160		6.3		139		x		30		35		3
161		5.8		140		xx		25		64		6
162		6.8		136		x		28		100		Yes
163	4.6		137		xxx		60				o	
164	4.9		139		x		79		50		2	
173	6.5		135		xxx		50		10		6	
178	3.0		135		x		100		5		6	
179	6.6		136		xxx		30		50		3	
181	3.9		137		xxx		50		50		3	
182		4.5		136		o		15		10		Yes
183	3.5		138		xx		30				2	
184		5.6		136		o		45		40		11
185		4.1		137		o		45		45		Yes
186	5.1		137		xxxx		100				1	
196		4.2		135		o		50		70		Yes

Number of experiments in *Groups I* and *II*:

	Group I: 22	Group II: 39	Total: 61
Survival, permanent:	o	29 (74%)	29 (47%)
Survival, 1 to 36 hours:	20 (91%)	10 (26%)	30 (50%)
Survival, less than 1 hour:	2 (9%)	o	2 (3%)

giving back all of the blood removed.   Whether this was in part counteracted by the ouabain we can only guess.

The tetraethyl ammonium ion, following the early work of Burn and Dale (20), has recently been proved by Acheson and Moe (21) to block transmission of impulses over autonomic ganglia.

Ten years ago, Freeman *et al.* (23) published an important paper in which they demonstrated that after total sympathectomy, blood pressure could be reduced to lower levels and for longer times without producing shock than in normal animals. But they were unable to tolerate as large hemorrhages.   The difference in reaction was correlated with the peripheral blood flow.   In normal dogs, as the blood pressure was reduced by hemorrhage to 70 mm. Hg, blood flow was reduced below 2 ml. per minute, while in the sympathectomized animal it was above 2 ml.   The preferential treatment of blood supply to vital centers is lost in sympathectomized animals, but as long as these centers receive sufficient blood supply, all the tissues of the body probably receive an adequate amount of blood and shock is prevented.   H. C. Wiggers *et al.* (22) also have demonstrated a deleterious effect of sympathetic over-activity by blocking with dibenamine.   This appeared to delay the onset of terminal shock.   For these reasons, it was of particular interest to determine the effect on the response to hemorrhage of blockade of all autonomic ganglia.

The animals, 51 in number, were subjected to the shock procedure and either before or within one hour of bleeding given 10 mg/kg. body weight of tetraethyl

ammonium chloride intravenously. A sharp fall in blood pressure occurred, especially when it was given before bleeding. But even after bleeding, the injection

TABLE 4. SURVIVAL RATE AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION AND OUABAIN (LATE OUABAIN EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
221		3.4		135		x		90				Yes
223		3.8		135		x		100		100		30
230		6.2		135		x		38				11
232		9.3		135		x		100		50		Yes
234		4.7		161		o		100		80		Yes
236		5.4		139		x		77		80		Yes
238		4.8		143		o		37		100		Yes
239	3.1		148		XXX		100		5		10	
240	6.1		273		XXX		83		5		10	
241		4.8		145		x		50		87		Yes
243	4.4		135		XXX		50		o		10	
258		5.7		137		x		100		70		Yes
270		4.8		137		o		75		75		Yes
292		6.3		143		x		62		37		10
295		5.0		143		x		70		30		Yes
296		5.2		143		o		38		56		Yes
297		7.6		163		o		32		60		Yes
298		6.4		149		o		50		108		Yes
299		6.5		180		x		50		37		Yes
300		6.2		153		o		36		154		Yes
302		6.2		159		o		45		100		Yes
305		5.3		185		x		100		57		Yes
306		6.4		144		x		32		50		Yes
308		5.4		162		o		65		80		10
309		5.5		160		x		50		12		10
310		6.3		147		o		90		160		Yes
311		7.6		145		o		44		42		Yes
312		5.8		139		o		55		42		Yes
313		6.2		145		o		36		37		Yes
418		5.9		144		x		94		83		Yes
423		4.8		149		x		100		50		Yes
424		6.3		180		o		39		60		Yes
429		5.0		174		x		72		83		Yes

Number of experiments in *Groups I* and *II*:

	Group I: 3	*Group II:* 30	Total: 33
Survival, permanent:	o	25 (83%)	25 (76%)
Survival, 1 to 36 hours:	3 (100%)	5 (17%)	8 (24%)
Survival, less than 1 hour:	o .	o	o

elicited a considerable fall in arterial pressure which was compensated for by the influx of about 23 per cent of the total amount of withdrawn blood over periods averaging 16 minutes. The response to intravenously injected adrenalin was greatly

augmented by the tetraethyl ammonium as Page and Taylor (25) had shown. Re-transfusion was always followed by an injection of ouabain. Survival data are presented in table 5.

Almost all of the animals, 23 out of the 24 with favorable prognosis, survived.

TABLE 5. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION, OUABAIN AND TETRAETHYL AMMONIUM CHLORIDE

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
323		5.5		145		o		90		150		Yes
325		7.0		141		x		100		112		Yes
327		6.7		145		o		71		270		Yes
328		6.2		172		x		47		50		Yes
329	6.6		158		x		100				10	
330		6.6		165		x		66		100		Yes
331		5.7		143		x		71		72		Yes
332	5.0		143		xx		69		120		Yes	
336		6.0		160		o		62		96		Yes
338		5.2		162		o		68		50		Yes
339		4.3		156		x		75		178		Yes
340		5.8		142		x		46		55		Yes
341		5.3		201		x		48		110		Yes
342	5.1		145		xx		83		51		Yes	
344	6.0		158		xx		75		94		Yes	
345		4.8		181		x		62		48		10
346		5.4		151		x		48		53		Yes
347	6.5		141		xx		44		50		Yes	
348	5.6		140		xx		100		100		10	
349		7.7		148		x		100		133		Yes
350		5.6		150		o		50		41		Yes
351	4.0		144		xx		84		150		Yes	
352	6.3		140		xx		44		124		Yes	
353		5.9		160		o		41		100		Yes
354		7.4		164		o		64		134		Yes
356	5.6		149		xx		46		80		Yes	
357		5.1		139		o		53		83		Yes
358	4.6		152		xx		63		73		Yes	
359		4.4		171		x		87		145		Yes
360		5.0		152		x		38		80		Yes
369	4.8		141		xx		40		55		10	
370	4.0		139		xx		34		45		10	
425		4.8		140		x		90		81		Yes
426		8.4		204		o		77		67		Yes
427		5.8		238		o		75		210		Yes
428		3.4		172		x		67		75		Yes
440	3.8		139		x		83		100		15	
441	3.7		142		xx		100		100		8	
442	5.9		153		x		30		100		Yes	
443	3.6		145		x		96		60		6	

TABLE 5.—*Continued*

I EXP. NO.	II BLEEDING VOL., ML./100 GM. B. VT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ANDRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
444	5.3		149		x		78		88		8	
445	5.1		147		xx		71		100		Yes	
448	6.0		135		xx		44		43		15	
449	5.4		141		xx		33		90		Yes	
450	2.8		145		xx		64		100		8	
451	4.1		139		xx		33		38		12	
452	4.6		141		xx		56		132		8	
459	4.0		154		xx		100		67		12	
461	8.3		162		x		78		100		Yes	
467	5.2		145		xx		70		200		8	
468	5.8		142		x		33		63		8	

Number of experiments in *Groups I* and *II:*

	Group I: 27	Group II: 24	Total: 51
Survival, permanent:	12 (44%)	23 (96%)	35 (69%)
Survival, 1 to 36 hours:	15 (56%)	1 (4%)	16 (31%)
Survival, less than 1 hour:	0	0	0

Of the 27 dogs with unfavorable prognosis 44 per cent also survived permanently. This is in sharp contrast to the 45 control animals with unfav rable prognosis (tables 1–4) which were treated with intra-arterial transfusion alone or with ouabain and of which only 2 survived.

The long, and as yet unexplained, sequence of animals with bad prognosis mentioned previously and evident in the experiments given in table 5 starting with no. 440 (February 1948) affects the average results toward less rather than greater survival.

Despite this, it is obvious that tetraethyl ammonium chloride produced an increase in survival rates which occurred whether the outlook for survival was favorable or unfavorable. It is probable that the temporary transfusion of almost one fourth of the withdrawn blood volume, which the fall of arterial pressure after administration of tetraethyl ammonium chloride caused, had some effect upon the survival rate. However, this is probably not the sole explanation for similar transfusions given purposely to animals with unfavorable prognosis in other shock experiments at comparable periods during hypotension had no effect upon survival rate.

Comparison of the survival rates obtained in all our experiments with those of other investigators (2–5), using much the same technique to produce hemorrhagic shock, shows them to be similar in our 'early' group of experiments but markedly higher in the late control group and notably in the groups given ouabain or tetraethyl ammonium chloride. The chief difference in treatment seems to have been that other workers returned the blood by vein while we employed the artery. The advantages of retrograde intra-arterial transfusion have been reviewed elsewhere (17). In our experiments it was invaluable.

In contrast with intravenous transfusion, arterial pressure can be restored to

normal exceedingly rapidly. The volume of blood transfused is automatically determined by the pressure-volume requirements of the vascular tree. Further, less blood is needed to achieve and maintain comparable arterial pressures.

It was of interest to follow the course of the infused blood in a dog in which circulation had all but failed, especially because Kohlstaedt and Page (6, 7) had noted the fact that patients who had stopped breathing often took a deep breath within seconds of administration of blood into the femoral artery. So quick was this reaction that the blood would not have had time to complete the circuit of the body and so reached the hypoxic respiratory center. With this in mind, x-ray photographs
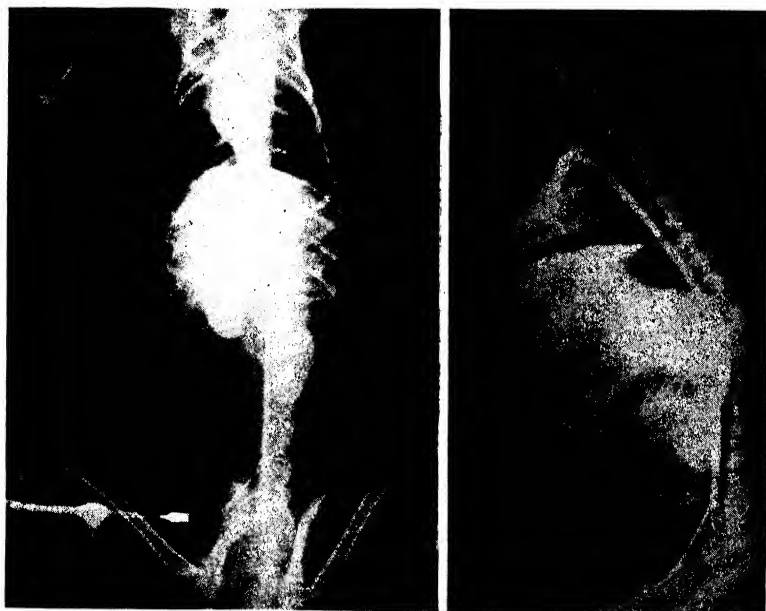
Fig. 5. INTRA-ARTERIAL INFUSION of Skiodan into the femoral artery of a dog deeply in shock with extreme hypotension. *Left.* Note that the kidneys fill first. *Right.* Same animal a few seconds later. The coronary and medullary vessels have now filled.

were taken serially as a 70 per cent solution of Skiodan was infused into the femoral artery of a dog almost dead from severe bleeding.

Figure 5 was taken a few seconds after the infusion was started and shows that the kidneys fill first, followed by filling of the coronary and spinal medullary vessels. Thus retrograde flow up the aorta occurs when the arterial pressure is low.

The rapid, controlled restoration of arterial blood pressure can be of decided importance in an emergency, many of which we encountered during the course of these experiments. With blood pressure at 30 mm. Hg, a further fall can be disastrous unless quickly countered by arterial transfusions. Intravenous administration is often too slow under such circumstances. To further prove this point in a series of experiments, we deliberately dropped the pressure to zero by withdrawing, after the shock procedure, further quantities of blood. This led to stoppage of circulation and

TABLE 6. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK AND RESUSCITATION

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.	III TIME OF HYPOTENSION, MIN.	IV REL. INTAKE DURING HYPOTEN.	V INTAKE DURING TRANSF., % OF BLEEDING VOL.	VI ADRENALIN RESP., % OF ORIG. RESP.	VII SURVIVAL
244	5.2	166	xx	70	50	10
245	4.9	168	x	90	0	36
246	5.4	161	o	100	30	Yes
247	5.8	138	xxx	100	100	Yes
248	6.2	179	x	100		0
250	6.2	170	xx	60	20	10
251	7.0	163	o	50	20	10
253	5.9	170	x	70	10	10
254	5.5	158	xxx	75	0	3
256	4.6	178	xx	100	100	9
259	5.0	198	x	100		0
261	7.0	167	x	100		0
262	3.4	167	x	66	65	Yes
264	5.1	152	x	75	40	10
265	7.1	154	o	40	0	10
267	5.8	225	o	87	90	Yes
268	6.3	163	o	65	75	10
271	6.0	151	xxx	82	40	3
272	6.7	176	o	60	50	Yes
274	4.0	162	x	100	0	10
275	4.4	167	o	66	5	Yes
276	7.3	190	o	54	26	Yes
277	6.9	168	· o	73	44	Yes
278	3.9	175	x	100		0
281	5.2	145	o	43	40	10
282	5.7	191	x	100	50	10
283	6.9	145	o	32	33	10
291	8.4	163	o	100		0
293	5.4	167	o	100	23	10
294	6.3	168	x	100	50	Yes
318	6.0	163	xx	63	83	10
320	8.1	173	xx	57	40	10
321	8.6	157	o	58	54	Yes
325	7.0	141	x	100	100	Yes
326	4.8	136	o	53	30	Yes
333	5.8	163	x	100	100	Yes
334	4.8	166	x	100		0
335	5.5	183	x	100	56	10
337	4.5	155	x	50	75	10

No. of expts: 39. Survival, permanent: 13 (33%); survival, 1 to 36 hrs.: 20 (51%); survival, less than 1 hr.: 6 (16%).

respiration, i.e., a condition where resuscitation by means of intravenous transfusion did not promise success.

In 39 dogs already subjected to the usual hemorrhagic shock procedure, more blood was withdrawn into the pressure reservoir, until the heart and respiration stopped as indicated by electrocardiographic and pneumographic records.

Respiration usually stopped first and was started artificially in from 2 to 8 minutes. When the heart had stopped for about 2 minutes, treatment was started. This consisted of rapid intra-arterial transfusion along with 0.5 ml/kg. of 1:10,000 adrenalin, artificial respiration and ouabain (0.05 mg/kg.) intravenously. The results are presented in table 6.

Eighty-four per cent of the animals could be resuscitated by this method; 51 per cent of these lived for an average period of 10 hours and 33 per cent survived indefinitely. Thus 16 per cent failed to be resuscitated, which seems to us a small number in view of the long period of hypotension followed by stoppage of heart and respiration. An example of the records of these experiments is presented in figure 6.
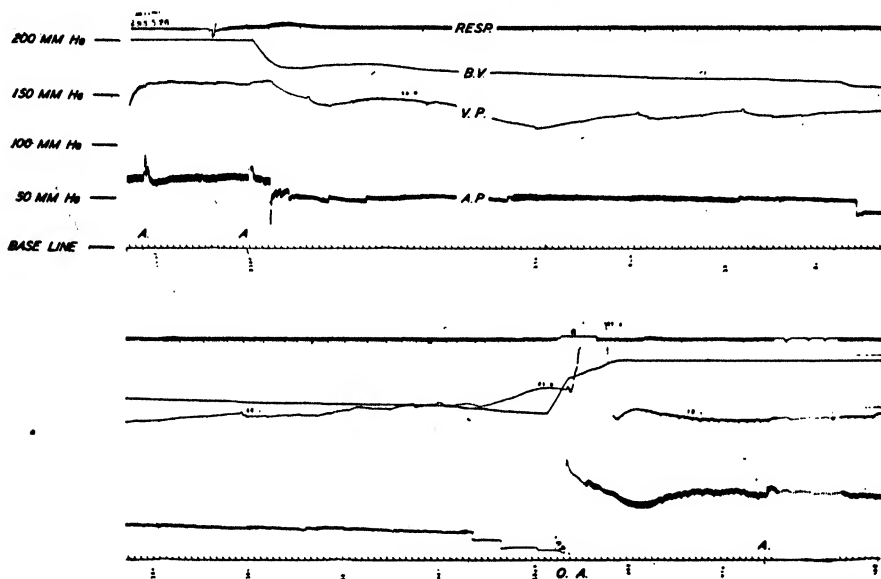
Fig. 6. RECORD OF DOG resuscitated following shock procedure and further bleeding until respiration stopped for 6 min. and the heart (as measured by the electrocardiogram) for 2½ min. Total time of the hypotension was 167 minutes. Venous pressure (V.P.) is also recorded from the right auricle.

While we have no control observation on animals, it seems reasonable to assume that arterial transfusion and artificial respiration were the chief factors in the resuscitation under these conditions of emergency. Obviously, an *intravenous* transfusion could not have been of benefit. It is important to point out that enough heparin has entered the animal's (24) circulation to reduce the possibility of coagulation and the blood returned to the animal was heparinized providing an additional safeguard against intravascular clotting.

SUMMARY

The effect of hemorrhage of severe grade and intra-arterial retransfusion on the survival of 244 dogs was studied. It was found possible, confirming our earlier work, to divide the animals into those with good and poor outlook for survival. The criteria selected were 1) maintenance of a steady pressure during the hypotensive period without takeup of blood from the constant pressure reservoir, 2) a relatively

small amount of the blood withdrawn being required to restore the normal level of arterial pressure and 3) no marked change in responsiveness to adrenalin after re-transfusion as compared with the control.

During the period covering about the first year of the work, the survival rate for those with poor prognosis was 7 per cent and with good prognosis 35 per cent. The next two years saw a marked increase in the number of animals with good prognosis, from 35 to 84 per cent with permanent survival with a corresponding decrease in those with bad prognosis. Survival in the latter group did not increase significantly. Periods of weeks to months occurred when for no reason we could discover, survival fell off sharply only to recover without change of technique, a phenomenon we had previously noted in scalded animals.

Administration of ouabain did not affect the survival rate in 94 animals, except possibly in the early group of experiments where over-transfusion may have adversely affected survival in the animals with good prognosis. Tetraethyl ammonium chloride significantly increased survival, 44 per cent in animals with bad prognosis and 96 per cent with good.

The value of intra-arterial transfusion in emergency is emphasized. Thirty-nine animals after being subjected to the shock procedure were bled further until breathing and heart beat stopped, the former from 2 to 8 minutes, the latter about 2 minutes. Eighty-four per cent could be resuscitated, 51 per cent lived an average of 10 hours and 33 per cent survived indefinitely.

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MECHANISM OF THE ARTERIAL PRESSURE RESPONSE TO THE VALSALVA TEST: THE BASIS FOR ITS USE AS AN INDICATOR OF THE INTACTNESS OF THE SYMPATHETIC OUTFLOW

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THE details of the response of arterial pressure to short periods of high intrapulmonary pressures (40 mm. Hg) have not previously been clearly elucidated. It is the purpose of this communication to define the reflex pathways over which this response travels and to indicate its usefulness as a test for the degree of activity of the sympathetic nervous system, in the normal and after sympathectomy.

Since Valsalva first described the procedure of sustained expiratory effort against a closed glottis (1), the test has been used for various purposes.[1] Several years ago, it was determined in the dog that the marked rise in blood pressure following the release of a high intrapulmonary pressure did not occur if an appreciable reduction of blood volume had previously been brought about (fig. 1). At that time, the response to the Valsalva test was used as an index of the adequacy of the circulating blood volume (2). In anticipation of the study of shock patients, control data were obtained from patients with normal blood volumes on the surgical wards of the Massachusetts General Hospital. The rise in blood pressure that followed the release of a high intrapulmonary pressure was found to be present in all but the sympathectomized patients. The following experiments resulted from this observation.

Certain phases of the arterial pressure response to forced expiration have been carefully studied by Wilkins and Culbertson (3). These authors found that in man, following the Valsalva test, the overshoot of femoral arterial pressure was either diminished or abolished after bilateral sympathectomy. Direct proportionality was not established between the degree of sympathetic denervation and the diminution of the overshoot in any given patient, although the collected data suggested that such a proportionality might exist.

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[1] The various names given to this test or maneuver (clinically) are likely to give rise to confusion in regard to understanding its mechanism. It is certain that simply drawing in a deep breath and holding it does not initiate that sequence of events resulting in a uniform post-stimulus overshoot. The term 'breath-holding test' would, therefore, seem to be inadequate. Drawing in a deep breath and blowing it out as forcefully as possible is likewise not likely to yield either a striking or consistent response. Actually the proper test (as was first described by Valsalva) is a sustained, forced, expiratory effort against an obstructed airway which is usually either a closed glottis or a column of mercury. It is suggested, therefore, that the test be called either by Valsalva's name as heretofore, or the term 'forced obstructed expiration' be used.

METHODS

Male and female dogs weighing from 9 to 15 kilograms were used.   Morphine sulphate (5 to 10 mgm.) was used as preliminary medication, after which urethane was administered as needed for quiet steady anesthesia.   This amounted to between 0.4 and 1.0 grams per kilogram of body weight.   No anesthetic agent was administered between tests which were being compared to each other.

Arterial pressure was recorded from the femoral artery.   The pressure-recording device used was an electronic strain gage led through a carrier type amplifier to a direct-writing oscillograph[2] as previously devoloped (4).   The paper on which all the tracings in this experiment were made was printed in millimeter divisions.   The paper speed for all experiments was 2.4 mm. per second.   Intravenous, intracaval, intra-auricular and intraventricular pressures were recorded through a no. 9 catheter 100 cm. long.

In some experiments an incision was made in the atlanto-occipital membrane and a no. 6 ureteral catheter threaded down the subarachnoid space until the tip
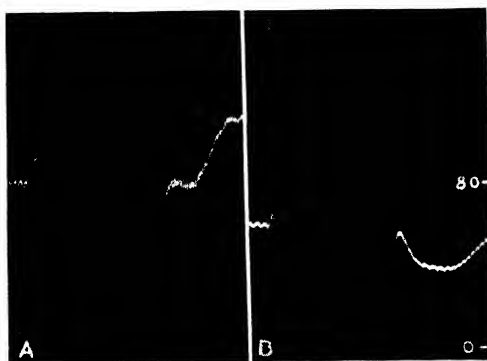
Fig. 1. ARTERIAL PRESSURE RESPONSE to intratracheal pressure of 40 mm. Hg applied for 30 seconds.   Stimulus *A* was applied prior to bleeding.   Stimulus *B* was applied following the removal of one sixth of the dog's estimated blood volume.   Blood pressure in mm. Hg.   Time marker indicates 5-second intervals.   Dog is under nembutal anesthesia.

reached the sacral area.   Varying doses of 1 per cent procaine hydrochloride could then be conveniently administered.   As demonstrated by Co Tui (5) a total spinal anesthesia can be induced by this catheter technique.   Co Tui's method was modified to produce a graded and also reversible chemical sympathectomy, since the subsequent washing out of the procaine in the subarachnoid space with saline was capable of removing the block and restoring the sympathetic influence on the periphery in a matter of minutes.

A tracheal cannula was secured in place and connected to a source of compressed air.   This stream of air under pressure was run through a mercury trap so that the pressure in the system would always be at, but never exceed, 40 mm. Hg.   The trap was connected to a 20-liter carboy in such a way that a large reservoir of air under a pressure of 40 mm. Hg would be available to afford an instantaneous stimulus.   A side arm on the tracheal cannula allowed the dog to breathe normally between stimuli.

An ordinary sphygmomanometer cuff was wrapped snugly, but not tightly, around the dog's chest with its upper margin in the axillae.   The dog was then

[2] Sanborn Company, Cambridge, Mass.

secured to the dog board in the supine position. A rubber tube from the cuff was attached to the source of compressed air from the carboy so that the perithoracic cuff would be inflated at the same time and by the same pressure that was applied to the lung.

The standard stimulus, used in every case, was as follows. At the onset of the stimulus, the airway was closed and the tube from the pressure reservoir opened, thus exposing the lung and perithoracic cuff simultaneously to a pressure of 40 mm. of Hg. After 30 seconds, the airway was opened and the tube leading from the pressure reservoir clamped shut, permitting immediate restoration of normal conditions of breathing. Three hundred and fifty-seven such tests were performed on 10 dogs.

When vagus section was performed, it was done high in the neck. When tetraethylammonium chloride was used, 100 mgm. in 10 cc. of saline were injected into the femoral vein in a period of about one minute.

Fig. 2. Typical response of femoral arterial pressure to the Valsalva test. *Components a, b, c, d, e* and *f* explained in text. Pressure in mm. Hg at right. Signal marks indicate beginning and end of applied intratracheal pressure which lasted for 30 seconds.

RESULTS

Response to Standard Stimulus. Figure 2 is representative of the arterial pressure response to the standard stimulus as described above. It can be seen that immediately following the application of the standard stimulus there is a slight rise in pressure (a), followed by a rapid fall (b) and narrowing of the pulse pressure. There is then a tendency to level off (c) with a further narrowing of the pulse pressure. Immediately after the release of intrathoracic pressure there is a slight further fall (d), and then a bounding pulse wave that climbs to levels (e) far in excess of the prestimulus figure. This component (e) will be termed the overshoot. After a variable period of time, the blood pressure returns to its prestimulus level (f).

Figure 3 shows the response of the pressure in the femoral artery, femoral vein, abdominal vena cava, right auricle and right ventricle to elevation of intrapulmonary pressure to 40 mm. Hg for 30 seconds. All tests in figure 3 were done in succession on the same dog. The vagi had previously been cut.

All but the pressure tracing from the right ventricle are self-explanatory. The irregularities in the curve during the forced expiration on all the tracings correspond in part with the dog's attempts to make respiratory motions. These same irregularities are sometimes seen in the femoral artery tracings but are less well marked.

The right ventricular pressure response to forced expiration (fig. 3E) is of some additional interest in that there was a distinct overshoot in the right ventricle follow-

ing the release of intrapulmonary pressure just as in the systemic arterial response. The significance of this will be discussed later.

Effect of Vagus Section on the Overshoot.   Most of the experiments in this investigation were performed after the vagi had been cut.   This was done for two
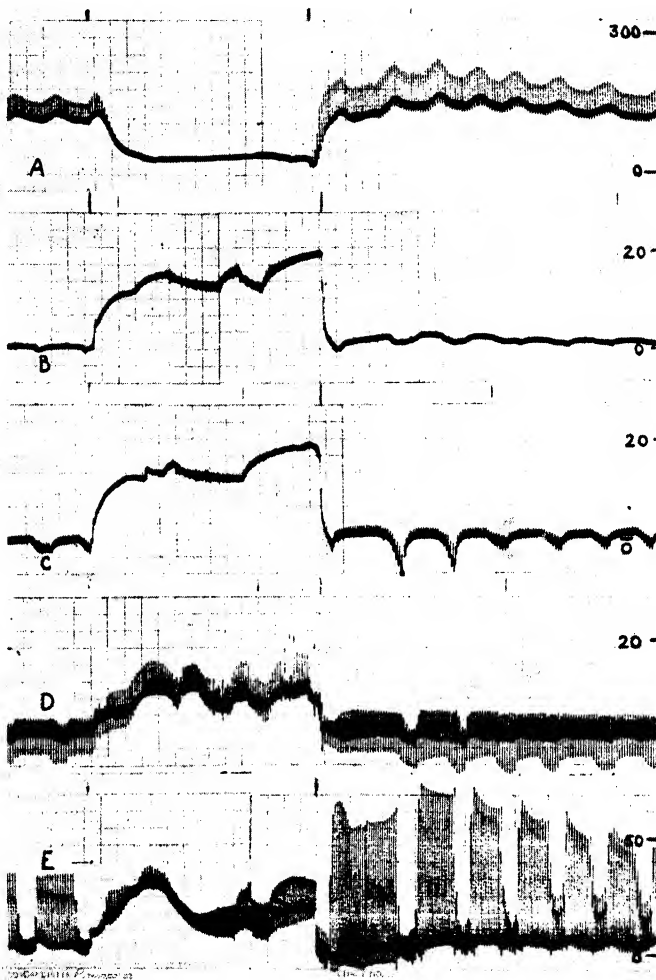
Fig. 3.   RESPONSES TO THE VALSALVA TEST of the pressures in: *A*, femoral artery, *B*, femoral vein, *C*, inferior vena cava, *D*, right auricle and *E*, right ventricle.   Pressure in mm. Hg designated to the right of each scale.   The various ranges were obtained by changing the amplification of the current from the strain gage to suit the particular need.

reasons.   First, the sectioning of the vagi yielded a steadier preparation and one free from the excessive vagal activity that usually accompanies morphine-urethane anesthesia.   This made interpretation and comparison of the records easier and gave larger overshoots with which to deal, since after section of the vagi the extent of the overshoot usually increased and became more uniform.   This fact led to the

conclusion that vagal activity can inhibit the extent of the overshoot.   Figure 4A shows the overshoot response with intact vagi, and figure 4B, the response after the vagi had been cut.   It can be seen that the overshoot response after vagus section is greater.   Atropinization had the same effect.   Secondly, it was thought that by cutting the vagi, the aortic depressors would be eliminated and a more satisfactory study of the influence of the carotid sinuses could be made.

Effect of Spinal Anesthesia on the Overshoot.   Figure 5 shows the effect of gradually ascending spinal anesthesia on the overshoot (*component e*).   The vagi had previously been cut.   The response prior to spinal anesthesia is shown in figure 5A, where a distinct overshoot is seen.   The responses obtained in the same animal, as the spinal anesthesia is driven gradually higher by additional injections of procaine, are shown in figure 5B, C and D.   It can be seen that as the block ascends and the blood pressure falls, the overshoot diminishes and is finally abolished.   In figure 5E is shown the effect of the rapid irrigation of the spinal canal with saline and the rise
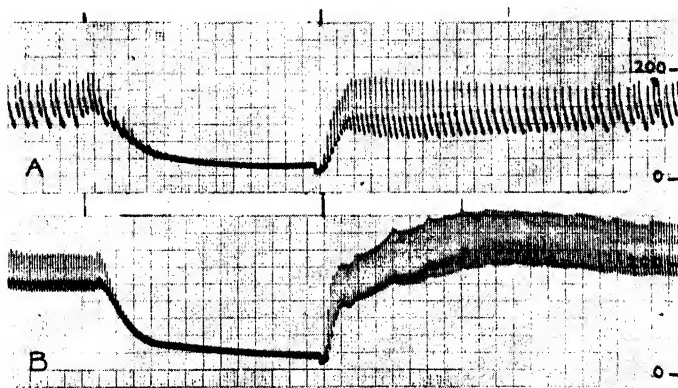
Fig. 4. Effect of cutting the vagi on the arterial pressure response to the Valsalva test. *A*, prior to vagotomy; *B*, two minutes following vagotomy.   Blood pressure in mm. Hg at the right.

of blood pressure to normal levels.   In 5F the overshoot is again present in a test obtained after the washing.   These data are representative of the results of this procedure in three dogs, in which no contrary data were obtained.

Effect of Tetraethylammonium Chloride on the Overshoot.   Figure 6 shows the effect of tetraethylammonium chloride on the femoral arterial response to forced inflation of the lungs.   Acheson *et al.* have demonstrated that this agent blocks autonomic impulses at the ganglionic synapse (6, 7).   Since the vagi had previously been cut in this experiment, the effect of the drug is presumably on the sympathetic outflow.   Figure 6A shows the response prior to the administration of the drug. Figure 6B shows the fall of blood pressure directly after the injection of 100 mgm. of tetraethylammonium chloride intravenously.   In figure 6C it can be seen that the overshoot has been greatly diminished.   In other experiments it was abolished. Figure 6C, D and E show the responses during and after recovery from the drug. These figures are representative of the results of this procedure in 5 dogs, in which no contrary data were obtained.
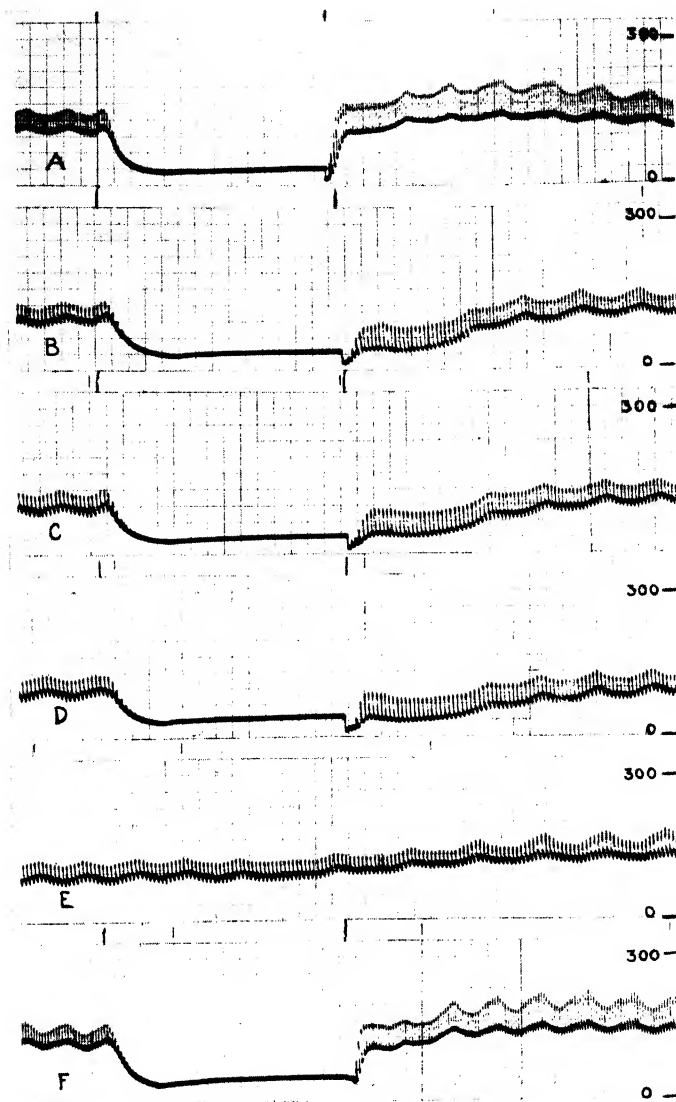
Fig. 5. EFFECT OF GRADUALLY ASCENDING SPINAL BLOCK on the arterial pressure response to the Valsalva test. *A*, normal response. *B*, response 11 minutes after injection into spinal catheter of 30 mgm. (3 cc.) of procaine; between *B* and *C* (6-minute interval) second injection of procaine, 10 mgm. (1 cc.). *C*, response 5 minutes after second procaine injection; between *C* and *D* (6-minute interval) third injection of procaine, 10 mgm. (1 cc.). *D*, response 3 minutes after third procaine injection. *E*, prompt rise in blood pressure accompanying the washing out of procaine from the spinal canal with 100 cc. of normal saline injected into catheter (signal mark in tracing *E* indicates beginning of wash). *F*, response 10 minutes after completion of spinal wash. Blood pressure in mm. Hg at right.

Effect on the Overshoot of a Continuous Intravenous Infusion of Epinephrine. It was thought that the effect of a continuous infusion of epinephrine might shed some

light on the efferent pathway involved in the overshoot response to the Valsalva test. For, if the overshoot were due to sympathetic activity reflexly engendered during the period of forced expiration, in the presence of excess circulating epinephrine the
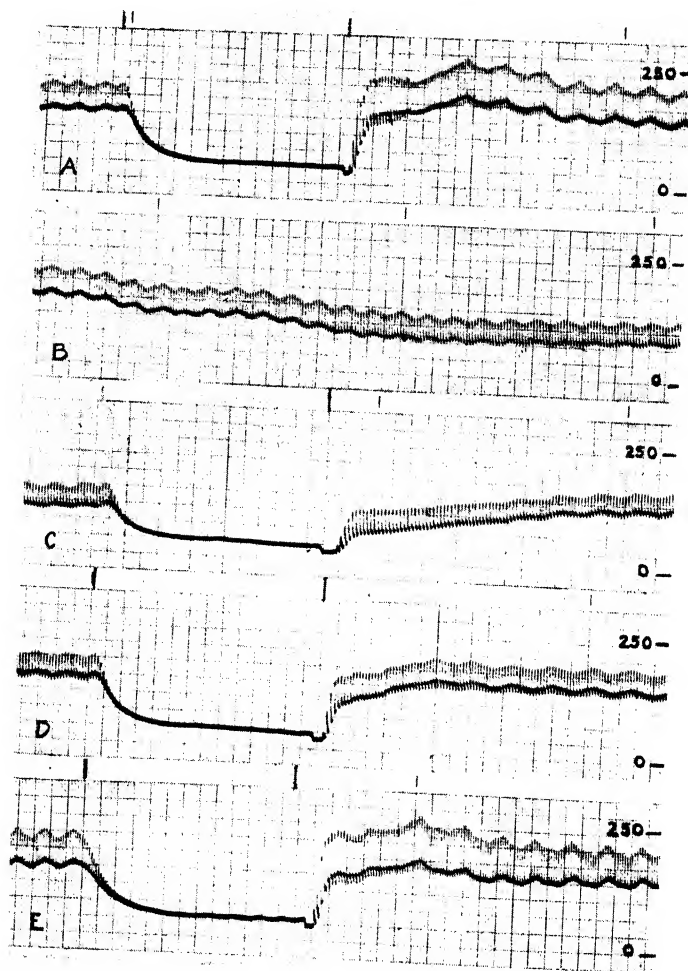
Fig 6. EFFECT OF INTRAVENOUS TETRAETHYLAMMONIUM CHLORIDE on the arterial pressure response to the Valsalva test. *A*, normal response; *B*, fall in arterial blood pressure following the intravenous injection of 100 mgm. of tetraethylammonium chloride (10 cc.) given at beginning of tracing; *C*, response 2 minutes after injection; *D*, response 6 minutes after injection; *E*, response 1 hour after injection. Blood pressure in mm. Hg at the right.

stimulus should find the efferent pathway already activated and, therefore, have little or no additional effect.   Figure 7 shows the effect on the overshoot response of a continuous intravenous infusion of 0.1 cc. per kilogram per minute of a 1:10,000 solution of epinephrine hydrochloride.   Figure 7A shows the response prior to the

infusion; figure 7B the response during the infusion.   It can be seen that prior to the infusion the overshoot is appreciable and during the infusion it is abolished. Figure 7C shows the response after the epinephrine infusion was stopped, and it can be seen that the overshoot has returned.   These figures are representative of several tests that were done before, during and after the infusion.   The largest overshoot obtained during the infusion was always smaller than the smallest overshoot obtained before and after the infusion.

Effect of Common Carotid Artery Occlusion on the Overshoot.   Figure 8 shows the effect on the overshoot of occluding both common carotid arteries.   The vagi have abolition of the overshoot response during carotid occlusion.   Figure 8C shows the
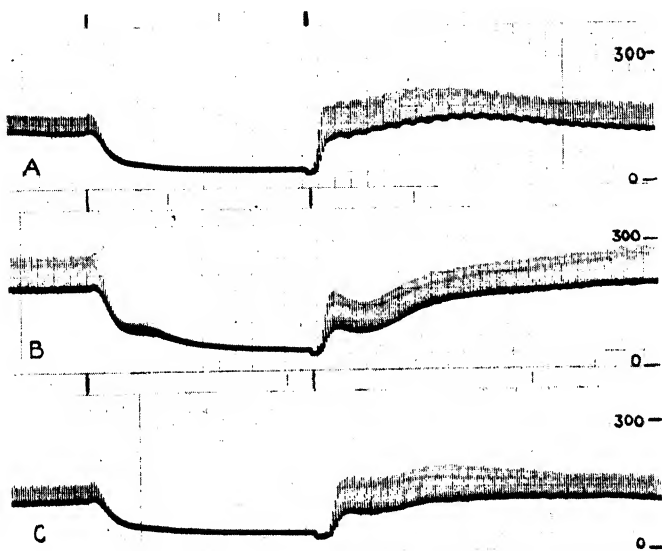
Fig. 7. EFFECT OF CONTINUOUSLY ADMINISTERED intravenous epinephrine hydrochloride on the arterial pressure response to the Valsalva test.   *A*, response prior to infusion; *B*, response during a continuous infusion of 0.1 cc/kgm/min. of a 1:10,000 solution of epinephrine hydrochloride; *C*, response 11 minutes after stopping the infusion.   Blood pressure in mm. Hg at the right.

been cut.   Figure 8A shows the response with open carotids.   Figure 8B shows the overshoot after release of the carotids; it can be seen that the overshoot has regained its initial extent.   These data are representative of the results of this procedure in 8 dogs in which no contrary data was obtained.   The overshoot was not abolished in every case but was always significantly diminished.   This was observed in the intact (fig. 9) as well as the vagotomized dog.   In those experiments in which the overshoot was diminished but not abolished by carotid occlusion, it was thought that a possible explanation might be as follows.   The pressure in the carotid sinuses is lowered by carotid occlusion yet is high enough to be further affected (via vertebral anastomoses) by the systemic hypotension resulting from the test stimulus, thus
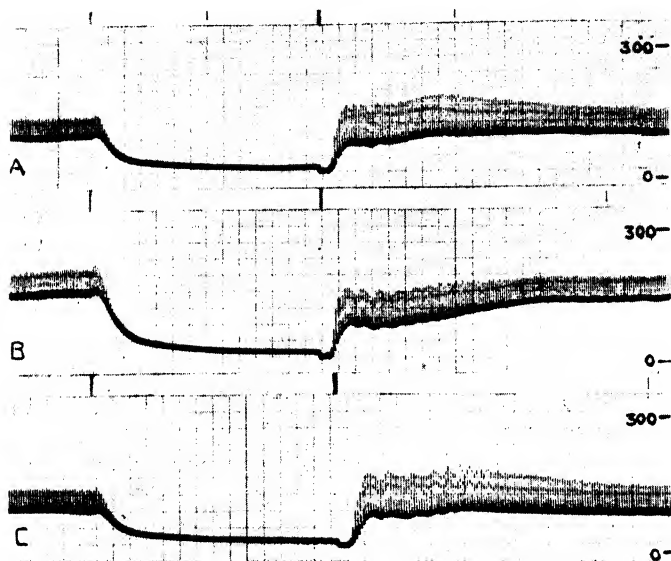
Fig. 8. EFFECT OF CAROTID ARTERY OCCLUSION on the arterial pressure response to the Valsalva test. Vagi cut. *A*, response prior to carotid occlusion; *B* response during occlusion of both common carotid arteries; *C* response after release of the carotids. Blood pressure in mm. Hg at the right.
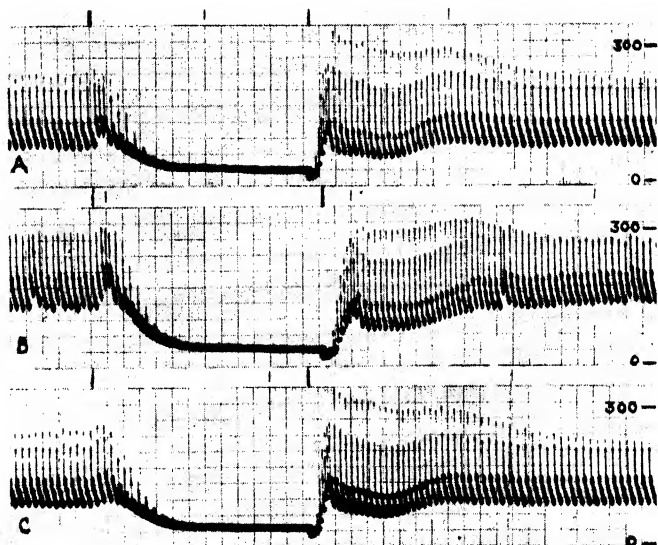
Fig. 9. EFFECT OF CAROTID ARTERY OCCLUSION on the arterial pressure response to the Valsalva test. Vagi intact. *A*, response prior to carotid occlusion; *B* response while both common carotid arteries are occluded; *C* response after release of the carotids. Blood pressure in mm. Hg at the right.

bringing into play more vasoconstrictor activity. This was verified by direct observation.

DISCUSSION

Although factors affecting the height of the overshoot of blood pressure following the Valsalva test are the important considerations in this group of data, the explanation of the other components of the blood pressure curve during and after the application of increased intrapulmonary pressure will be briefly discussed first.

Component a. Immediately after the intrapulmonary pressure is increased there is a slight but definite rise in arterial pressure in some instances. At the time the stimulus is applied, the pulmonary vascular tree has a normal amount of blood in it. This sponge-like structure is then squeezed by the sudden thrust of air against the chest wall that is kept more or less fixed by external pressure. The contained blood is thus forced in the direction of least resistance, namely towards the left heart, yielding a sudden increment in left venous return, cardiac output, and, thus, an increased systemic arterial pressure. This appears to be the most logical explanation for the *a* component, even though in some dogs no *a* component was ever elicited.

Component b. The precipitous fall *b* is due to a diminution in venous return. An increase in intrapulmonary and intrathoracic pressure is produced and acts like a pneumatic clamp across the vessels of the pulmonary bed and the great veins in the thorax. The blockade must be almost complete and the result is a marked decline in venous return, cardiac output and systemic arterial pressure.

Component c. Following the precipitous fall *b*, there is a tendency for the arterial pressure to level off *c* at very low levels.

Component d. Immediately following the release of pressure in the airway there is a further slight fall in arterial pressure *d*. The compressed vascular tree of the sponge-like lung expands, momentarily absorbing right ventricular output, and thereby momentarily decreases venous return to the left ventricle, left ventricular output and systemic arterial pressure.

Component e. The bounding rise in arterial pressure to heights far in excess of prestimulus levels is the significant component in the clinical test for the degree of intactness of sympathetic vasoconstrictor pathways. The marked overshoot does not occur in the absence of the vasoconstrictor mechanism as evidenced by the experiments with spinal anesthesia and tetraethylammonium chloride. Likewise, the activity of the carotid sinuses is essential, since, if they are excluded, the response is either abolished or diminished. It seems logical to postulate, therefore, that the overshoot is a result of the following sequence of events: *a*) When the intrathoracic pressure is raised at the onset of the stimulus, the arterial pressure falls sharply and the carotid and aortic pressor receptors are exposed to greatly diminished intraarterial pressures; *b*) the vasomotor center responds to the decreased afferent impulses and reflex pressor mechanisms are brought into action via vasoconstrictor, adrenal and cardio-accelerator fibers. If these pathways are intact, the onrushing blood released at the end of the stimulus is put out of the left ventricle more forcefully against an intensely constricted peripheral arteriolar bed and the pressure rises to extreme heights. On the other hand, if a significant portion of the peripheral vasoconstrictor mechanism is not functioning, the blood is put out against an arteriolar bed to which the vasoconstrictor impulse has not been fully applied, and the arterial

pressure does not exceed prestimulus levels if the defect in the efferent sympathetic pathway is severe enough. The extent to which the adrenal glands participate in this response has not been determined, but they might reasonably be expected to play an appreciable part. The effect of the release of accumulated metabolites on the circulation during the overshoot period has not been evaluated.

The overshoot of right ventricular pressure was at first thought to be due solely to the release of blood that had been dammed back on the venous side of the circulation during the stimulus. This interpretation would be in keeping with the experiments of Euler and Liljestrand in which carotid artery occlusion had little or no effect on the pulmonary arterial pressure of the cat under chloralose (8). It was with some surprise, therefore, that we found first a marked increase in the level of right ventricular pressure following simple bilateral common carotid artery occlusion (fig. 10). A systematic investigation of this interesting reflex response has been undertaken and will be reported separately. Secondly, the magnitude of the right ventricular overshoot was appreciably diminished if the test was performed while the carotids were occluded. This led us to believe that occlusion of the common
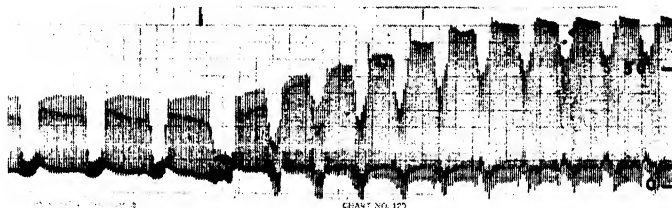
Fig. 10. EFFECT OF BILATERAL COMMON CAROTID ARTERY OCCLUSION on right ventricular pressure. The occlusion was performed at the signal. Blood pressure in mm. Hg at the right.

carotid arteries has a specific effect on right ventricular systolic pressure, elevating the resting level and also decreasing the magnitude of the overshoot response.

The effect of spinal anesthesia on the arterial pressure response to the Valsalva procedure should be evaluated in the light of recent work on the rôle of the sympathetics in the hypotension induced by spinal anesthesia. It had previously been thought that under spinal anesthesia muscular stasis might be responsible for the hypotension. However, the results of differential blocking procedures (in which the sympathetic but not the motor neurones in the subarachnoid space were blocked) indicated that paralysis of the sympathetic outflow was largely, if not completely, responsible for the fall in blood pressure under spinal anesthesia (9-12).

The extent to which extraneous factors can alter the overshoot must be considerable. It has been shown that a low blood volume can diminish or abolish it (2). Factors which cause greater than normal sympathetic activity in the prestimulus period (such as cold, fear, pheochromocytoma etc.) might be expected to alter the overshoot by pre-exciting the efferent sympathetic pathways before the test is performed. Either excessive vagal activity or medication interfering with vagal action might alter the overshoot by either augmenting or diminishing the normal

inhibitory action of the vagus. Likewise, hypersensitivity of the carotid sinuses logically falls in that group of causes that might be expected to alter the response to the Valsalva test.

SUMMARY

1. Pressure changes in various parts of the circulatory system, resulting from a 30-second elevation of intrapulmonic pressure, form characteristic and consistent patterns.

2. The arterial pressure response has six components, of which the poststimulus overshoot, or rise of blood pressure above the prestimulus level, is the main subject of this paper.

3. This overshoot is the result of reflex sympathetic activity engendered by the hypotension present in the carotid sinuses during the period that venous return is impaired. (The extent of participation by the aortic receptors was undetermined.)

4. Removal or diminution of carotid sinus activity diminishes or abolishes the overshoot.

5. Partial or complete blockade of reflex sympathetic activity either by tetra-ethylammonium chloride or by graded, segmental, spinal anesthesia diminishes the overshoot *in direct proportion to the extent of the blockade.*

6. Vagal activity inhibits the overshoot to a variable degree.

7. The presence of an excess of circulating epinephrine diminishes and may abolish the overshoot.

8. The overshoot of right ventricular systolic pressure is influenced by the presence or absence of carotid sinus activity, much as is the overshoot of systemic arterial pressure.

9. The standardization of any clinical test designed to use the degree of over-shoot as a quantitative estimate of sympathetic activity should take into considera-tion the factors of medication, vagal activity, cold, apprehension, carotid sinus sensitivity and the presence of circulating epinephrine, as well as the patient's blood volume.

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CARDIAC EFFECTS OF INTRAVENOUS INJECTION OF SMALL VOLUMES OF STRONGLY HYPERTONIC SOLUTIONS[1]

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THE intravenous injection of even small volumes of strongly hypertonic solutions may result in marked circulatory changes in mammals. Numerous studies have been carried out in an attempt to analyze these effects, particularly with reference to the therapeutic uses of such injections. Among the vascular and cardiac changes which have been described in cats, dogs and human beings are generalized vasodilatation (1–3), coronary arterial constriction (4), alterations in the electrocardiogram, and tachycardia (3, 5). We have noted in a series of experiments on cats and dogs that the effects of such injections upon the heart may be more complex than described previously. The cardiac alterations include irregularities, dropped beats or extrasystoles, and, perhaps most characteristically, a precipitous and profound slowing of the heart, which is abolished by bilateral section of the vagus nerves. The latter effect may be demonstrated with ease in cats and, less readily, in dogs as well. The purpose of this paper is to describe in some detail the cardiac effects of the intravenous injection of small volumes of strongly hypertonic solutions.

METHODS

Experiments were performed on 20 cats ranging in weight from 1.7 to 4.0 kg. and 13 dogs varying from 3.5 to 15 kg. in weight. Most of the animals were anesthetized by the intravenous or intraperitoneal injection of nembutal (sodium pentobarbital 36 mg/kg.). In addition, experiments were carried out on 3 dogs which had been decerebrated under ether anesthesia, upon one unanesthetized (local 2% procaine anesthesia), and on 2 chronically sympathectomized dogs.

In general, simultaneous femoral arterial pressure tracings were made using a standard recording mercury manometer and a Hamilton metallic membrane manometer with optical recording. Heart rates and information as to the general character of the pulse waves were obtained from the Hamilton manometer records. The error in calculating the heart rate from counts of the number of pressure pulses seen on the Hamilton record, during successive three-second intervals, was estimated as being of the order of ±5 per cent. The mercury records were used to relate the mean arterial pressure with the observed cardiac rate changes. Electrocardiogram records were obtained by the use of a General Electric portable electrocardiograph.

The effects of the injection of 20 per cent sodium chloride solution were studied in detail. In a few additional experiments, records were also taken during the intravenous administration of other hypertonic solutions including 5, 10 and 15 per cent NaCl and 50 per cent glucose. The volumes of these fluids given in a single injection ranged from one to 6 ml. for cats, and from 2 to 16 ml. in the observations upon dogs. All of the solutions were at room temperature. Equal volumes of 0.9 per cent NaCl were given in several experiments as controls on the mechanical and thermal effects of this type of fluid infusion. The results of injecting comparable volumes of distilled water and of approxi-

mately isotonic solutions of low and of high pH (0.155 M. hydrochloric acid and 0.155 M. sodium hydroxide) were studied also.

Injections were made into the external jugular vein, or less frequently, into the femoral vein or one of the auricular appendages which had been exposed by opening the chest during intermittent positive pressure ventilation of the lungs. Solutions were administered as rapidly as possible through 20-gauge needles inserted into rubber-capped cannulae tied into the blood vessels. The rates of injection varied from one to 4 ml. per second and were timed accurately by closing a key in a circuit so that a light flashed on the Hamilton record and a signal magnet writing on the mercury record was activated. In the later experiments, the key was fixed upon the end of the plunger of the injection syringe and thus was pressed automatically at the start of the injection. Using the injection signal obtained in this manner on the manometer records, the latency of the response to any given solution was measured as the time from the beginning of the injection to the beginning of the first cardiac cycle of a series showing prolongation.

RESULTS

Observations on Cats. In 7 of 12 experiments on cats, transient cardiac irregularities were observed within a second after the start of the injection of 2 to 3 ml. of 20 per cent NaCl. The irregularities were seen on the Hamilton records as
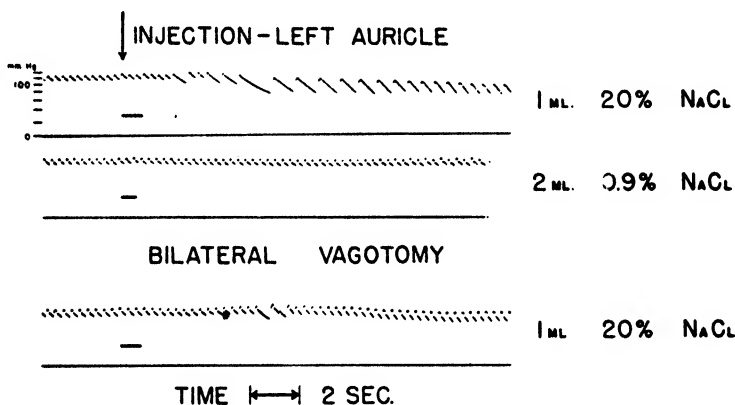
Fig. 1. *Cat 12.* NEMBUTAL ANESTHESIA. Femoral arterial pressure records showing the characteristic bradycardia following the rapid injection of 20% NaCl and its absence after bilateral vagotomy. All injections into cannulated left auricular appendage.

shortened cardiac cycles with an occasional cycle of unusually long duration. Immediately after this brief period of irregularity, or occurring even in its absence, was a far more consistent and striking cardiac change. This was marked slowing of the heart, as illustrated in figure 1 from a typical experiment. Such bradycardia was noted in all experiments upon nembutalized cats, as shown by analysis of both Hamilton manometer and electrocardiographic records. It commenced within 1.8 to 4.0 seconds (av. 2.34 ± 0.78 sec.) following intrajugular injection and in three experiments resulted in complete cardiac arrest for three to nine seconds. In other instances the minimum heart rates observed, which occurred within 3 to 21 seconds after injection into the external jugular vein, varied between 22 and 63 per cent of the control rate. A transient and moderate to marked lengthening of the PR interval (by 20 to 100% of the duration of the control PR interval) was noted at this time in the three experiments in which ECG records were obtained. The bradycardia lasted for an average of 107 seconds, the duration ranging in all experiments

from 36 seconds to 6 minutes. At the end of this period, the heart rate returned to or surpassed the pre-injection level. Thus in four experiments transient tachycardia, in which the heart rate rose to a peak 12 to 20 per cent higher than the control rate, followed the phase of bradycardia. It may be noted at this point that the mean arterial pressure fell more or less parallel with the decline in heart rate. The arterial pressure did not return to the control level as rapidly as did the heart rate, and the slight tachycardia seen several minutes after the injection of hypertonic NaCl may have resulted from reflex adjustments to the concomitant hypotension.

Following bilateral cervical vagotomy, the pattern of heart rate changes in response to 20 per cent NaCl injection was altered markedly (fig. 1). Usually some cardiac irregularities occurred after the injection, but true bradycardia was not observed in any of the experiments after the vagi had been sectioned. In three experiments a slight and brief decrease in heart rate was noted, yet this cardiac slowing was of a totally different order of magnitude from the effect with the vagi intact. The duration was not more than three to nine seconds, and the slowing was of relatively lesser degree, the lowest rate observed under these conditions being 80 per cent of the control rate. Marked alterations in the PR interval were not noted after bilateral vagotomy. On the other hand, slight but significant tachycardia occurred in five experiments following the injection of 20 per cent NaCl in vagotomized animals. In two cases the heart rate increased to 26 and 31 per cent of the control, and the elevation in rate lasted for 30 and 45 seconds, respectively. In spite of the fact that marked bradycardia did not occur, the mean arterial pressure decreased in vagotomized cats. Usually the hypotension was of moderate degree, however, the fall in pressure averaging 42 mm. Hg less than the fall occurring when the vagi were intact. In only one experiment was the fall in mean arterial pressure found to be greater after than before vagotomy, when identical volumes of 20 per cent NaCl were injected into identical regions of the vascular bed.

The control injections of isotonic (0.9%) NaCl produced negligible changes in heart rate in both vagotomized and intact animals. In seven experiments, the heart rate was observed to vary within ± 11 per cent of the control level during the first 15 to 18 seconds after the injection, a period during which striking bradycardia always occurred after injection of the strongly hypertonic solutions. In four experiments a transient and questionable rise or fall in mean arterial pressure (± 5 to 6 mm. Hg) was noted, but it lasted only during and for one to two seconds after the injection.

Table 1 summarizes the results of six experiments in which hypertonic solutions other than 20 per cent NaCl were injected. These solutions included 15 and 10 per cent NaCl and 50 per cent glucose (approximately isotonic with 10% NaCl). That the bradycardia observed was a result of the hypertonicity of the injected fluid, rather than a specific effect of excess concentration of sodium (or chloride) ion upon the heart may be concluded from the fact that the injection of 50 per cent glucose caused marked bradycardia just as did hypertonic NaCl. The response to the former solution was somewhat less striking, but it was quite comparable quantitatively with the cardiac slowing following the injection of 10 per cent NaCl. Thus, in some animals marked decrease in heart rate followed the injection of 2 ml. of 20

per cent NaCl, whereas no bradycardia was seen after the injection of 2 ml. of 10 per cent NaCl or 50 per cent glucose. In these animals, however, the heart rate slowed after injection of twice this volume of 10 per cent NaCl or 50 per cent glucose. It may be noted from table 1 that 15 per cent NaCl regularly produced bradycardia which was qualitatively similar to, though less marked than the slowing following injection of 20 per cent NaCl. In preliminary tests it was found that injection of 5

TABLE 1. COMPARISON OF THE EFFECTS OF THE INTRAVENOUS INJECTION OF VARIOUS HYPERTONIC SOLUTIONS IN ANESTHETIZED CATS

EXPER. NO.	SOLUTION INJECTED	VOL.	SITE OF INJECTION	RATE OF INJECTION	VAGAL BRADYCARDIA		
					Latency	Max. degree[1]	Duration
		ml.		*ml/sec.*	*sec.*		*sec.*
4	20% NaCl	1	L. J. V.	1.5	—	Absent	—
	"	2	"	1.5	2.3	46	28
	"	2	"	0.4	4.0	62	19
	50% Glucose	4	"	1.3	4.5	50	24
29	20% NaCl	2	Rt. Aur.	1.2	1.8	13	34
	15% NaCl	2	"	3.3	0.9	5	16
	10% NaCl	2	"	2.0	—	Absent	—
	"	4	"	4.2	1.2	3	22
	50% Glucose	2	"	2.5	—	Absent	—
	"	4	"	4.7	0.8	3	21
55	20% NaCl	2	L. J. V.	2.9	2.0	2.6	19
	15% NaCl	2	"	2.7	1.6	1.8	13
	10% NaCl	2	"	2.4	—	Absent	—
	50% Glucose	4	"	2.9	2.7	1.9	20
56	20% NaCl	2	L. J. V.	1.8	2.1	2.1	26
	15% NaCl	2	"	1.9	3.2	1.2	12
	10% NaCl	2	"	2.4	—	Absent	—
	50% Glucose	2	"	1.7	—	Absent	—
	"	6	"	2.1	1.4	1.4	25
57	20% NaCl	3	L. J. V.	2.1	2.4	2.8	26
	15% NaCl	3	"	2.3	—	Absent	—
	10% NaCl	3	"	2.3	—	Absent	—
	50% Glucose	6	"	2.6	—	Absent	—
58	20% NaCl	3	L. J. V.	2.1	3.0	35	48
	15% NaCl	3	"	2.3	3.0	20	55
	10% NaCl	3	"	2.5	4.4	1.2	13
	50% Glucose	6	"	1.3	—	Absent	—

[1] Maximal degree of vagal bradycardia = duration of most prolonged cardiac cycle following injection ÷ duration of average control cardiac cycle.

per cent NaCl did not result in bradycardia. Specific tests of the effects of varying the rates of injection of the hypertonic solutions were not made. It may be noted, however, that volumes of 10 and 20 per cent NaCl which caused marked bradycardia when administered rapidly could be introduced very slowly and caused no alteration in heart rate. In general, it would appear that the concentration, volume and rate of injection of hypertonic solutions are interrelated variables determining the occurrence and intensity of the bradycardia response.

Of the other fluids tested, distilled water, a 'maximally hypotonic solution', did not alter the heart rate in the quantities used (2 to 4 ml.). Similarly one ml. volumes of the strongly alkaline solution, approximately isotonic sodium hydroxide, were without effect. The intravenous administration of one ml. of isotonic hydrochloric acid caused a characteristic and totally different type of response in the five experiments in which it was tested—delayed hypotension, apnea, and occasionally a slight bradycardia. It may be concluded then that the injection of highly hypertonic solutions produces a profound slowing of the heart rate in anesthetized cats with vagi intact and that this effect is quite specific and dependent upon the increased concentration of the injected fluid. It has been observed also that such injections may result in cardiac irregularities even after bilateral vagotomy. In addition, there is transient hypotension which is exaggerated by the concomitant vagal bradycardia, but occurs even in the absence of any change in heart rate. The overall response is, however, more complicated than this. Alterations in the depth and rate of respiration occur and will be described in more detail in a subsequent paper. The vari-

TABLE 2.   VAGAL BRADYCARDIA IN UNANESTHETIZED AND DECEREBRATED DOGS RESULTING FROM THE INTRAVENOUS INJECTION OF 20 PER CENT SODIUM CHLORIDE

EXPER.		VOL.	RATE	VAGAL BRADYCARDIA		
				Latency	Max. Degree[1]	Duration
		ml.	*ml/sec.*	*sec.*		*sec.*
989	Unanest.	8	2.7	3.8	1.5	7
		12	2.6	5.0	12	30+
D1	Decer.	8	2.9	3.0	8	7
D2	Decer.	4	1.8	4.5	38	82
D3	Decer.	3	2.1	5.3	3	8

[1] Maximal degree of vagal bradycardia = duration of most prolonged cardiac cycle following injection ÷ duration of average control cardiac cycle.

ations in cardiac rate, mean arterial pressure and respiration appear to be independent to a large extent and, yet without doubt, each is modified by the others through reflex cardiovascular and respiratory adjustments.

Observations on Dogs. The results obtained in the seven experiments which were carried out on normal dogs anesthetized with nembutal were in marked contrast to the effects seen in nembutalized cats. Instead of the consistent and profound bradycardia dependent on the presence of intact vagi, as described in the latter, the alterations in heart rate were inconstant. In three experiments the heart rate was slowed by less than 11 per cent and a comparable degree of bradycardia occurred after bilateral vagotomy. Similarly, some decrease in heart rate was noted after vagotomy in two of the four experiments in which irregularities with more marked slowing of the heart had occurred when the vagi were intact. In these four experiments, the minimum heart rates observed ranged between 37 and 77 per cent of the control rates. In most of the experiments there was a profound fall in arterial pressure and, closely correlated with this, a tachycardia which occurred within 30

to 36 seconds in six of the seven experiments upon normal nembutalized dogs.   Such tachycardia was absent in one of the two experiments carried out on chronically sympathectomized dogs anesthetized with nembutal.   In the other sympathecto-mized animal, however, tachycardia was observed even after bilateral vatotomy and may, therefore, be tentatively ascribed to direct action of the hypertonic agent on the heart.

As they stand, these results indicate that the vagal bradycardia following the injection of hypertonic solutions, as seen in cats, does not occur in the dog.   It has been shown, however, that anesthesia with barbiturates reduces the cardio-in-hibitory action of the vagi in mammals (6, 7), and consequently four additional experiments were carried out upon dogs under conditions such that anesthetic effects were eliminated as far as possible.   The experiments were conducted on one un-anesthetized dog (local procaine anesthesia) and three preparations in which de-cerebration had been performed under ether anesthesia, and the ether subsequently removed.   In all of these experiments, marked bradycardia was elicited by the rapid injection of 3 to 12 ml. of 20 per cent NaCl.   In latency, duration and intensity, this bradycardia was quite comparable to the cardiac slowing seen in cats following ad-ministration of hypertonic solutions (table 2).   The bradycardia was, moreover, abolished by bilateral cervical vagotomy.   The injection of corresponding volumes of 0.9 per cent NaCl was found to be without measurable effect on the heart rate or arterial pressure.   Thus the specific response to the injection of hypertonic solutions was closely similar to the effects previously observed in cats.   As in the latter species, rather complex respiratory and arterial pressure alterations following the injection of hypertonic NaCl were noted as well.

DISCUSSION

Transient arterial hypotension following the injection of small volumes of strongly hypertonic solutions has been described by a number of investigators (1, 4, 8–11), but these workers have not noted the occurrence of marked vagal bradycardia in cats and dogs after such injections.   Sollman (12) cited Retzlaff as stating that the injection of larger volumes of hypertonic solutions caused a fall in blood pressure and speeding of the heart rate followed, in later stages by cardiac slowing as a result of stimulation of the vagus center.   On the other hand, Kisch (2) noted that in anesthetized cats the slowing of the heart, which resulted from injection of one cc. per kg. body weight of hypertonic salt and sugar solutions, was not mediated by the vagi since the pattern of circulatory changes was unaffected by vagotomy.   Bernstein (1) and Binet and Stoicesco (8) did not describe bradycardia in etherized and chloralosed dogs and Bernstein stressed rather the occurrence of tachycardia which he attributed to both vagal and sympathetic mechanisms. Gennari and Levi (10) found that one cc. of 20 per cent NaCl per kg. body weight injected into anesthetized dogs caused hypotension lasting for a period of 30 seconds or more and that this phenomenon was not affected by atropine or vagotomy.   Their published records give no evidence of bradycardia.   Similarly, Muirhead *et al.* (4) observed that the intravenous injection of 10 per cent NaCl, 50 per cent glucose and

25 per cent albumin in dogs anesthetized with sodium pentobarbital was without significant effect on the heart rate, although transient arterial hypotension occurred. The fact that such a constant and striking phenomenon as the vagal bradycardia described in the present paper has not been noted previously may, perhaps, be referred to the predominant use of anesthetized dogs as subjects for studies on the cardiovascular effects of intravenous administration of hypertonic solutions. Evidence from the experiments which we have performed on unanesthetized and decerebrated dogs as compared with nembutalized animals indicates that the occurrence of vagal bradycardia is abolished readily by anesthesia in the dog. It is far more easily demonstrated in the cat. At present, no evidence exists as to whether a similar phenomenon occurs in other species. It may be noted that it has not been described in anesthetized rabbits (13), nor in studies on unanesthetized human patients (3, 5). In the latter, ECG alterations and tachycardia may be observed to follow rapid intravenous injections of hypertonic solutions.

The significance of the vagal bradycardia described above is two-fold. On the one hand, a wide variety of hypertonic solutions—sodium chloride, glucose and other sugars, radio-opaque materials, etc.—are injected intravenously in certain clinical procedures and techniques of experimental physiology. Such infusions, if made at a sufficiently rapid rate, may have brief but profound cardiovascular effects which should be taken into account in carrying out the procedures and in interpreting the results of experiments in which they have been used. In addition the existence of this stable and characteristic response immediately raises the question of its physiological origin. Whether direct stimulation of the medullary vagus center occurs or whether the effect is attributable to reflex vagal excitation, it is highly specific in nature. Thus the response is dependent on increased concentration of the administered fluid, whereas alterations of tonicity in the opposite direction (distilled water) or of pH (isotonic acid and base) are without comparable results. Simple mechanical and thermal effects appear also to be excluded as factors participating in the origin of the response. An attempt has been made to establish the mechanism of the phenomenon and the results of this study will be presented in a subsequent paper.

SUMMARY

The intravenous injection of small volumes of strongly hypertonic solutions (10 to 20% NaCl, 50% glucose) results in marked alterations in the heart rate of cats and dogs. Cardiac irregularities occur initially and in nembutalized cats and unanesthetized or decerebrated dogs these irregularities are followed by transient but profound bradycardia which is absent after bilateral cervical vagotomy. This pattern of response is highly modified in dogs under nembutal anesthesia for, in these circumstances, relatively less or variable alterations in heart rate occur and true vagal bradycardia is absent. The species variation in the effects of nembutal upon this circulatory response mediated by the vagi obscures the fundamental similarity of the phenomenon in cats and dogs, for in both species profound vagal bradycardia can be demonstrated reproducibly under specifically defined conditions.

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MECHANISM OF VAGAL CARDIAC SLOWING FOLLOWING INTRAVENOUS INJECTION OF SMALL VOLUMES OF STRONGLY HYPERTONIC SOLUTIONS[1]

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AMONG the circulatory effects of the intravenous injection of small volumes of strongly hypertonic solutions, one of the most striking is a transient but profound bradycardia. Thus, within a few seconds after the rapid injection of 2 to 8 ml. of 20 per cent NaCl, 50 per cent glucose, or other highly concentrated solution into the veins of cats and dogs, the heart rate slows. This effect, which may last for several minutes, is abolished by bilateral section of the vagus nerves. It is present in nembutalized cats and in unanesthetized or decerebrate dogs, but not readily demonstrable in dogs anesthetized with nembutal (1). The experiments described in the present paper were carried out in order to analyze the mechanism of this vagal bradycardia as seen in anesthetized cats following the injection of strongly hypertonic solutions.

METHODS

Experiments were performed on 37 cats ranging in weight from 1.5 to 4.0 kg. Usually the animals were anesthetized with nembutal (36 mg/kg. intraperitoneally), but in a few experiments decerebration was performed under ether anesthesia and the ether was then removed. The trachea was cannulated and, in some experiments where intrathoracic structures were exposed, artificial respiration was administered by means of a small adjustable pressure pump. Simultaneous Hamilton manometer and mercury manometer pressure tracings were obtained from the two femoral arteries.

In general, heart rates were determined with an estimated error of ±5 per cent from the Hamilton manometer recordings by counting the number of cardiac cycles occurring during three-second intervals. In eight experiments, respiration was recorded by means of a chest pneumograph.

Injections of hypertonic solutions were made as rapidly as possible (1–4 ml/sec.) and timed accurately as described in a previous paper (1). The solutions used included hypertonic NaCl (15 and 20%) and glucose (50%). Control injections of isotonic (0.9%) NaCl were made as well. In a few experiments, sodium cyanide (0.4 to 2 mg. per kg. dissolved in 2 to 3 ml. of 0.9% or rarely 20% NaCl) was injected into the external jugular vein and the latency of the resulting gasp reflex was measured with a stop watch. The sites of injection of the hypertonic fluids included the external jugular veins, femoral veins, ascending aorta, and auricular appendages.

Experiments were carried out to determine whether the vagal bradycardia resulted from direct stimulation of the medullary vagus center, or from excitation of peripherally located receptors resulting in reflex vagal discharge. In the first series of experiments, a comparison was made of the occurrence and latencies of the responses following injection into peripheral veins, the heart, and the ascending aorta. Furthermore, the latent periods for the vagal bradycardia, following injection of 20 per cent NaCl and the gasp reflex resulting from NaCN injection into the same vein, were compared. In a second series of experiments, tests were made for the occurrence of vagal bradycardia after excluding the possibility of direct action of the hypertonic agent on the medullary

centers by isolation of the head from the arterial supply of the rest of the animal. Two techniques were used. In three experiments, pairs of animals of approximately equal weight were anesthetized and their chests opened while artificial respiration was maintained. The innominate and left subclavian arteries were doubly cannulated and connected by plastic tubing so that blood flowed from the ascending aorta of one cat into the innominate and left subclavian arteries of the other cat. Similarly, the innominate and left subclavian arteries of the first animal were perfused with blood from the ascending aorta of the second. In this way, the arterial supply of the head of one cat was obtained from the body of the other and the separate effects of injection of the hypertonic agent upon the body and upon the perfused head of each animal were tested. A second method for differentiating the peripheral as compared with the medullary effects of the hypertonic solutions was to expose and temporarily occlude the innominate and left subclavian arteries just prior to the intravenous injection of the hypertonic solution.

To determine whether the vagal bradycardia was dependent on afferent nerve fibers in the cardiac sympathetic nerves, four experiments were carried out in which injections were made before and after acute bilateral removal of the sympathetic chains from the stellate ganglia to T_7. In an attempt to analyze the rôle of the two vagi in the bradycardia, the left vagus only was sectioned in four experiments and injections were then made into the left atria. Subsequently the right vagus was sectioned and the results of hypertonic salt injection following this procedure were compared with the effects after unilateral vagotomy. Atropine sulfate (1–2 mg/kg.) was injected intramuscularly and the effect upon the vagal bradycardia was observed in six experiments. Finally, injections were made into 4 animals decerebrated under ether anesthesia, in order to determine whether any central nervous structures anterior to the medullary vagus center were necessary for the occurrence of bradycardia in response to the injection of hypertonic solutions.

It may be noted at this point that the vagal cardiac slowing can be abolished by non-specific factors, such as hypotension following opening of the chest, and the excessive manipulation of the lungs which may occur during removal of the thoracic sympathetic chains. When the technique for any given procedure was being developed, negative results were obtained occasionally, but these were not given the same weight as the positive findings obtained after the experimental techniques had been perfected. In the few experiments in which cardiac irregularities were observed, a response was considered to be true vagal bradycardia only if it involved the prolongation of many successive cardiac cycles and if it was abolished or greatly modified by bilateral vagotomy.

<div align="center">RESULTS</div>

In the 16 experiments in which hypertonic solutions were injected into various regions of the circulation, it was found that bradycardia occurred consistently after injections into the femoral and jugular veins and both right and left atria, but it was either not observed at all, or was seen only after a long latent period when injected into the ascending aorta. This is illustrated in figure 1, which reproduces the Hamilton manometer records from a typical experiment. It may be noted in this figure that the heart rate decreased following all injections of 20 per cent NaCl except for the injections into the ascending aorta. The control injections of 0.9 per cent NaCl were without effect on the heart rate. After bilateral vagotomy the hypertonic injections produced slight cardiac irregularities, but true bradycardia was absent. Table 1 presents a summary of the latencies of the vagal responses seen after injections into different regions of the circulation. In addition, the latent periods of the gasp reflex following the introduction of sodium cyanide into the external jugular vein are given in the table. The consistent occurrence of cardiac slowing after intravenous and intracardiac injections is notable, as is also the exceedingly brief latency (usually less than 2 sec.) of the responses following injections into the right and left atria. The latency of the bradycardia after left jugular injection (av.

2.3 sec.) was slightly longer and the response to femoral venous injection had a yet longer average latent period (4.9 sec.). In the single experiment in which brady-cardia was observed after intra-aortic injection, the latency was 3.9 seconds, or approximately twice the average latency of response to injections into the right and left atria. The briefness of the latencies of the bradycardia resulting from jugular
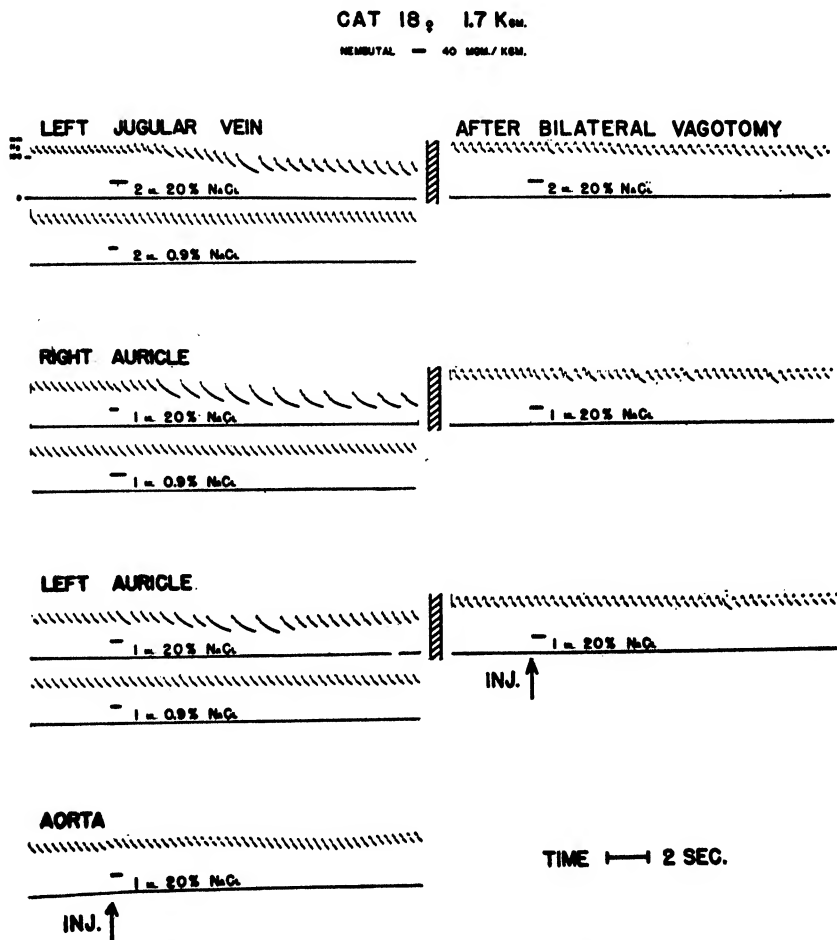
Fig. 1. *Cat 18.* NEMBUTAL ANESTHESIA. Femoral arterial pressure records showing the occurrence of bradycardia following the injection of 20% NaCl into the left jugular vein, the right and left auricles but not following injection into the ascending aorta. This response is abolished by bilateral vagotomy.

and cardiac injections suggests strongly that the structures stimulated by the hypertonic solutions are not as far from the site of injection as the medullary vagus center. This is further confirmed by comparison of the latency of the hypertonic salt bradycardia and the gasp reflex following cyanide injection. It may be seen from table 1 that the average latent period for the cyanide gasp reflex is 6.2 ± 2.1

seconds.   This latency probably represents, for the most part, the time required for the cyanide-containing fluid to pass from the jugular vein, through the right heart, the pulmonary vascular bed, left heart, and up to the carotid bodies (2).   If the brady-cardia which follows the injection of hypertonic solutions results from direct stimula-tion of the medullary vagus center, it would be expected that its latency would be longer than the latency of the cyanide gasp reflex.   That such is not the case is suggestive evidence that the effect of hypertonic solutions is not exerted directly upon the medullary vagus center.

As further and more direct evidence for this hypothesis, vagal bradycardia was elicited in two out of three experiments following intravenous injection of 20 per cent NaCl into animals in which the entire arterial supply to the head was obtained by cross-circulation from another animal, and in four out of six experiments when in-jections were made intravenously immediately after occluding the arterial supply to the head (innominate and left subclavian arteries).   It may be noted that in this series of experiments there was a fairly high proportion of negative experiments in which, after isolation of the head from the rest of the circulation, typical vagal bradycardia could not be demonstrated.   This may, perhaps, be attributed to poor

TABLE 1.   LATENT PERIODS OF VAGAL BRADYCARDIA AFTER INJECTION OF 1 TO 3 ML. OF 20% NACL INTO DIFFERENT REGIONS OF THE CIRCULATION AND OF THE GASP REFLEX FOLLOWING INTRAVENOUS INJECTION OF NACN

	LATENCY OF BRADYCARDIA AFTER INJECTION OF NaCl INTO				LATENCY OF GASP REFLEX AFTER INJECT. OF NaCN INTO LEFT JUGULAR VEIN sec.
	Femoral Vein sec.	Jugular Vein sec.	Right Atrium sec.	Left Atrium sec.	
Av.	4.9(3)[1]	2.3(18)	1.8(8)	1.6(8)	6.2(11)
Standard Deviation		0.9	0.3	0.7	2.1

[1] Numbers in parentheses indicate number of observations from which averages were obtained.

physiological condition of the medulla in these experiments, for the procedures used were, without question, somewhat radical.   In any case, the positive experiments, in which vagal bradycardia was observed after exclusion of the head from the circulation of the body, show that the characteristic bradycardia may occur quite independently of direct medullary excitation by the injected solution.

Since the hypertonic solution vagal bradycardia could not be attributed to direct stimulation of the medullary vagus center, it was concluded that it was reflex in character, resulting from excitation of peripheral receptors.   Injection into the left atrium usually resulted in marked bradycardia, but intra-aortic injection did not. This suggests that the receptors concerned are located, at least in part, within the heart itself and, more specifically, in the left heart.   As the latent period of the response was approximately the same regardless of whether the injections were made into the right or left sides of the heart, it seems improbable that the response from injection on the right side was completely dependent on receptors in the left heart, for this should lengthen the right side latent period by the duration of the pulmonary circulation time.   It is probable, therefore, that similar receptors are located in both the right and the left sides of the heart.

Figure 2, which represents data obtained from one of four similar experiments, illustrates the fact that bilateral removal of the sympathetic chains from the stellate ganglia to T_7 did not abolish the vagal bradycardia in response to hypertonic salt injection. Such partial sympathectomy should interrupt all cardiac afferent fibers running with the sympathetic nerves and, consequently, it was inferred that the afferent fibers from the receptors stimulated by the hypertonic solutions are not present in the sympathetic nerves and must be carried by the vagi. In four experiments, section of the left vagus nerve alone did not abolish, although it did reduce, the bradycardia resulting from the injection of hypertonic NaCl into the left atrium. This indicates that the vagal afferent fibers from the cardiac sensory endings must be bilateral in their distribution.

The intramuscular injection of atropine may completely abolish the vagal bradycardia. This result was obtained in two experiments on decerebrated cats
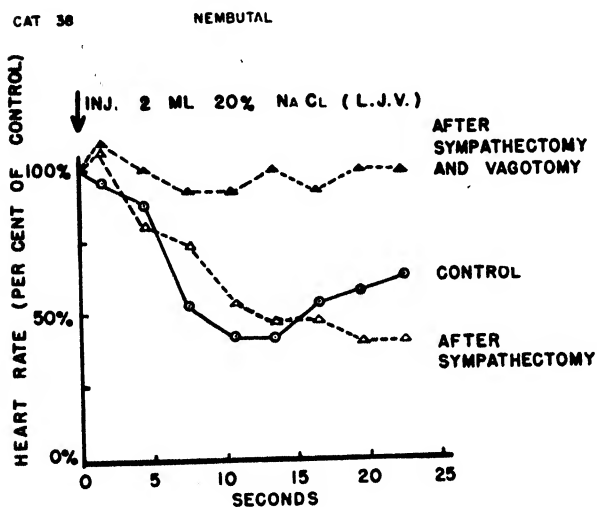
CAT 38   NEMBUTAL

Fig. 2. *Cat 38*. NEMBUTAL ANESTHESIA. Showing the occurrence of bradycardia before and after bilateral removal of the thoracic sympathetic chains from the stellate ganglia to T_7. Following bilateral cervical vagotomy, injection of 20% NaCl did not cause slowing of the heart rate.

injected with one mg. atropine sulfate per kg. and in cats under nembutal anesthesia injected with 2 mg. per kg. In additional experiments on 2 nembutalized animals which received one mg. per kg., the vagal bradycardia was not completely abolished, although it was markedly reduced. It was concluded from these experiments that there is no evidence for sympathetic participation in the observed bradycardia and that the efferent, as well as the afferent, path of the reflex arc is carried by the vagus nerves. Since it was found in four experiments that the vagal bradycardia could be elicited readily in the decerebrate preparation, the medullary vagus center must be adequate for completion of the reflex arc. It was concluded, therefore, that this reflex has the following neural components: a) receptors located, at least in part, within the left heart and probably on the right side of the heart as well; b) afferent fibers in the vagus nerves; c) the medullary vagus center; d) efferent fibers in the vagus nerves.

It may be noted that marked alterations in respiration occur concomitantly with the cardiac rate changes described. In general, these respiratory effects differed in character depending on whether the vagus nerves were intact or sectioned. In the animals with intact vagi, respiration was invariably arrested in expiration within a time interval ranging in eight experiments from 2.8 to 4.8 seconds (av. time, 3.4 sec.) after the injection. Usually the expiratory arrest preceded the characteristic fall in mean arterial pressure, whereas it succeeded by a fraction of a second the beginning of bradycardia. It lasted a variable length of time (3 to 43 sec.) and was followed, in general, by respiration which was irregular and frequently of increased depth. After vagotomy, expiratory arrest occurred in only one out of nine experiments, but respiration tended to become irregular and deepened, with prolongation and often exaggeration of the inspiratory phase. In general, these respiratory changes did not begin for seven seconds or longer and they were almost invariably preceded by the characteristic fall in blood pressure. Although the mechanism of the respiratory variations following hypertonic sodium chloride injection has not been analyzed in detail, the brief latency of the response and its occurrence preceding mean arterial pressure changes suggest strongly that it, too, is reflex in character. That such reflex modifications in respiration are dependent on afferent fibers running in the vagus nerves is suggested by the fact that the response is highly modified and frequently abolished by bilateral vagotomy. The slower and irregular changes noted after vagotomy may result from direct stimulation of the respiratory centers or from reflex stimulation arising from pressoreceptors of the carotid sinuses.

DISCUSSION

Evidence has accumulated that various chemical stimuli may excite intracardiac receptors with resultant vagal discharge. Jarisch and his co-workers (3–6) showed that the profound bradycardia and hypotension following the injection of several drugs (veratrine, mistletoe extract, histamine etc.), ions (potassium, rubidium etc.) and other substances may be attributed to the stimulation of intracardiac vagal receptors. It has been suggested by Jarisch and his collaborators that all of the agents found to cause this response (von Bezold effect) may produce cellular damage and release of ionic potassium. This potassium may be the fundamental stimulus for the observed bradycardia. A related phenomenon, perhaps, is the marked vagal slowing of the heart which results from the intravenous injection of homologous or heterologous serum in cats, as described by Brodie (7). The latter response was ascribed by Brodie to the stimulation of the pulmonary receptors of vagal afferents, but it resembles closely, in many respects, the responses described by Jarisch and the effect which we have observed. Our experiments do not answer directly the question as to whether the receptors, which mediate the response to hypertonic salt injection, are identical with previously described intracardiac sensory endings. It may be noted that the reflex effects resulting from veratrine injection, as described by Jarisch, included profound hypotension which was not entirely dependent on the bradycardia, since it occurred even after reflex cardiac rate changes had been abolished by atropinization. Such was not the case in our experiments, in which there was no consistent difference between the arterial blood pressure curves of

atropinized animals preceding and following bilateral vagotomy. It seems highly improbable that the receptors stimulated by hypertonic solutions are identical with the intracardiac stretch receptors described by Bainbridge and others (8, 9). Effective stimulation of these stretch receptors, located in the great veins and in the atria of both sides of the heart, results in reflex speeding of the heart rate. Exactly the opposite effect occurs following adequate stimulation of the receptors under consideration in the present experiments. Furthermore it may be re-emphasized that negative results following control injections of isotonic NaCl exclude the possibility of any mechanical component in this reflex.

The physiological rôle of the reflex patterns related to intracardiac receptors is not clear at present. It appears highly probable, however, that the heart, pulmonary bed and great veins may function in addition to the carotid-aortic chemoreceptor and pressoreceptor zones as regions of intravascular sensitivity to chemical and mechanical stimuli. The studies of Nettleship (10), Nonidez (11, 12) and others have provided histological evidence for a rich intracardiac receptor system in the mammal. Further, Amann and Schaefer (13) have demonstrated that centripetally travelling action potential bursts may be recorded from some of the cardiac branches of the vagi. Thus there is evidence both for the anatomical existence and for the physiological activity of intracardiac receptors. If their role is still far from being understood, it may not mean that they are of little physiological significance, but rather that the vascular adjustments which they mediate are delicate and occur in antagonism to, or in cooperation with, other reflex vascular changes.

SUMMARY

An analysis has been made of the mechanism of the profound vagal bradycardia following the intravenous injection of strongly hypertonic sodium chloride solutions in nembutalized and decerebrated cats. This vagal bradycardia does not result from direct stimulation of the medullary vagus center, but rather is of reflex origin. The receptors excited are located, at least in part, within the heart itself and the vagus nerves constitute both the afferent and efferent paths of the reflex arc.

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NERVOUS CONTROL OF THE CERVICAL PORTION OF THE ESOPHAGUS

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IN A recent communication (1) it was pointed out that in a dog after bilateral section of the vagus nerves just posterior to the level of the larynx the peristaltic activity of the upper third of the esophagus was preserved, as evidenced by balloon examination as well as roentgenological findings (fig. 1a, b). Consistent observations have been made by one of us (*K. H.*) in two more dogs. The current conception that the cervical portion of the esophagus is supplied by the recurrent laryngeal branches of the vagus would thus seem doubtful. The present study was therefore undertaken to investigate the specific motor supply to this portion of the esophagus in various animals.

Most modern textbooks of physiology and some monographs (2–7) state or imply that the recurrent laryngeal branch of the vagus is the motor nerve to the cervical portion of the esophagus. In some others either there is no definite statement (8) or it is simply said that the esophagus is supplied by esophageal branches of the vagus (9–11). All the statements in this regard are made without reference to the species of animal. Most of the old textbooks give no description of the nerve supply to the esophagus. But in the *Textbook of Physiology*, edited by Schafer (12), Starling stated that the upper part of the esophagus in man, rabbit and guinea pig is innervated by the recurrent laryngeal nerves, while in the horse and dog it is supplied mostly by a small branch of the pharyngeal nerve. However, he gave no reference to support the latter statement and no similar descriptions at all appear in the textbook written by himself (13).

The more recent workers are rather unanimous in the opinion that the cervical portion of the esophagus is supplied by the recurrent laryngeal branch of the vagus nerve and no mention is made about the earlier reports which held a view different from the current one. We were not aware of these discrepancies between the earlier reports and the more recent ones until the present experimental work had been almost concluded and the early literature was traced.

In 1836 Cooper (14) noticed distension of the esophagus with food material in rabbits after ligation of the vagus nerves in the neck. But the first extensive study of the relation of the vagus nerve to the esophagus was probably made by Reid (15) in 1838. He studied several species of animals and in the dog he noticed contraction of the pharyngeal muscles as well as the upper part of the esophagus on irritation of the pharyngeal branch of the vagus.

Chauveau (16a) made an extensive study in the horse, donkey, dog, lamb, cow and rabbit. He found that while the lower portion of the esophagus is supplied by the terminal branches of the vagus in all these animals, the upper portion is by the recurrent branches in the rabbit but in all the other animals by a long branch from the pharyngeal nerve, which, he states, was not known before. Brief descriptions of the innervation of the esophagus in different species also appear in his book (16b). He found (16c) that section of the vagi in the neck causes paralysis of the entire esophagus of the rabbit but only the lower portion in the dog and horse, while section of the 'superior motor nerve' of the esophagus in the horse involves the entire tracheal portion of the tube, which, however, does not affect deglutition seriously. Espezel (17) also noticed the difference of the innervation of the esophagus of the rabbit and the dog. He states that he is the first one to give a detailed description of the course of the 'inferior pharyngeal nerve', which is the motor supply for the upper portion of

the esophagus of the dog. He concluded that this nerve originates from the superior cervical sympathetic ganglion.

The same nerve was found in the dog by Kahn (18), but he prefers to call it the esophageal nerve. However, in regard to the origin of this nerve, he believed that it arises from the superior pharyngeal branch and also the nodose ganglion of the vagus nerve and received fibers from the superior cervical ganglion. He found in the cat, in accord with Reighard and Jennings (19), a pharyngo-esophageal nerve arising directly from the vagus trunk above the nodose ganglion or together with the superior laryngeal nerve. In the monkey (Macacus and Cynocephalus) he reported an esophageal branch from the superior laryngeal nerve. In all these animals he found, however, that the recurrent branch of the vagus gives motor innervation to a varying portion of the cervical part of the esophagus.

The results obtained by these workers in regard to the double innervation of the cervical portion of the esophagus have never been accorded the attention they deserve by the later investigators in this field. Kahn's work was quoted by Cannon (20, 21) and Inaoka (22) but received no confirmation nor comment regarding innervation of the cervical portion of the esophagus other than the recurrent. On the other hand, Inaoka's conclusion that the recurrent laryngeal nerve is the motor nerve to the cervical esophagus of the dog was supported by later workers (23–25). More recent work in the cat (26, 27) also is not in accord with Kahn's report.

Anatomists have noticed that the inferior pharyngeal branch of the vagus sends filaments to the upper part of the esophagus in the dog (28) and the cat (19), although another book (29) fails to describe them. In the monkey (Macaca mulatta) the cervical portion is believed to receive branches from neither the pharyngeal nerves nor the superior laryngeal nerve but from the recurrent (30). Recent work by Coulouma and Varseveld (31), based on dissection of 12 species of mammals, also made a general conclusion that the cervical portion of the esophagus is supplied by the recurrent or its branches.

Stoppage of barium meal in the upper part of the esophagus following extirpation of the superior cervical ganglion in the dog has been reported (32, 33). Knight (27) also observed in the cat that stimulation of the stellate ganglion increases the contraction of the upper third of the esophagus caused by vagal stimulation. However, Inaoka (22) concluded that the sympathetic nerves have no importance in the esophagus of mammals. Recent study in dogs after bilateral thoracic or complete sympathetic ganglionectomy has revealed no change in the activity of the esophagus (1).

EXPERIMENTAL

The early part of the present investigation was devoted to confirming in acute experiments that the recurrent laryngeal nerve is not essential to the activity of the cervical portion of the esophagus in the dog and to ascertain the real motor innervation.

Two preliminary experiments were made in dogs under nembutal anesthesia. The cervical portion of the esophagus was freely exposed by midline incision, resection of the sternohyoid and sternothyroid muscles and removal of a portion of the trachea, care being taken not to injure the recurrent laryngeal nerves. Faradic stimulation of the peripheral end of the vagus of either side resulted in strong tetanic contraction of the whole thoracic portion of the esophagus and the lowermost inch of the cervical portion. This is well evidenced by the fact that a finger put in the lower part of the esophagus through the cardia experienced during the vagal stimulation a strong grip by the esophageal musculature and simultaneously the cardia was passively pulled upward. This upward movement is in accord with the report by Rall, Gilbert and Trump (34); apparently the longitudinal and the circular fibers contract simultaneously. The cervical portion of the esophagus remained flaccid, showing no contraction of its musculature except its lowermost inch, and was mechanically pulled downward toward the thoracic cavity. Direct stimulation of the recurrent laryngeal

nerve at the base of the neck had no apparent effect on the esophagus although movement of the larynx was observed.

The findings above provided encouragement to proceed further to find the specific motor innervation of this portion of the esophagus. In the first experiment faradic stimulation of the superior laryngeal nerve on either side resulted in a weak but definite contraction of the whole cervical portion of the esophagus. In the second experiment no effect at all on the esophagus was observed by a similar procedure. Explanation for this discrepancy was found later. However, in this second dog faradic stimulation of a small nerve crossing the superior laryngeal nerve dorsally and then lying on the dorso-lateral aspect of the pharynx caused a very strong tetanic contraction of the whole cervical portion of the esophagus as well as the inferior pharyngeal constrictor muscle. The contraction was so strong that the cervical portion of the esophagus was, on stimulation of this nerve, immediately converted into a firm muscular cord comparable to the effect on the thoracic portion of the esophagus of stimulating the peripheral end of the vagus nerve. In the later experiments described below this nerve was consistently found and always manifested the same physiological action, and is believed to be the main, if not the only, motor innervation of the cervical portion of the esophagus in the dog. The anatomical course and the physiological significance of this nerve will be given below.

In all the experiments except the first two just described above ether anesthesia through tracheotomy was used. An audio oscillator (Hewlett Packard) was used for stimulation, the stimulating current used being adjustable in intensity and 10 to 50 cycles per second in frequency. This audio oscillator has the advantage of being adjustable to give a minimal effective stimulation, thus avoiding the effect due to the spread of the current. In some experiments the chest was opened along the midline of the sternum and artificial respiration was employed. The esophagus was then carefully exposed from the beginning at the level of the cricoid cartilage to the level of the diaphragm by excision of the lungs of the left side and division between ligatures of the left common carotid and subclavian arteries and then the aorta at the places where they cross the esophagus. Apparently there was no impairment of the excitability of the lower thoracic portion for at least one hour after the aortic transection, as judged by observations before and after this step of the operation. As a matter of fact, the slight depression of the animal as shown by the sluggishness of the corneal reflex after excision of the lungs on the left side, probably due to the resulting anoxemia, generally disappeared after the aortic arch was ligated. The beneficial effect obtained by this procedure is probably due to the improvement of the cerebral circulation. Similar experiments were done on the rabbit, cat and monkey.

In some dogs and cats chronic experiments were carried out by bilateral sectioning of this small nerve with sterile technique. Then roentgenological examinations by the aid of meals containing barium sulfate or balloon examinations as described in a previous communication were used (1).

1. *Anatomical Description of the Motor Supply to the Cervical Portion of the Esophagus*

Acute experiments have been done in dogs, cats, rabbits, monkeys, guinea pigs and rats. The course of the special motor nerve supply to the cervical portion of the

esophagus was mostly made out by careful dissection in the anesthetized animal with the help of electric stimulation. It should be emphasized here that, in general, the distal portion of the nerve overlying the esophagus is so closely attached to it and often breaks up into such fine branches that the actual length of the esophagus supplied by this nerve cannot be predicted precisely by means of gross dissection but only by electric stimulation. Furthermore, the anatomic course of this nerve may undergo some variations which also can only be ascertained by the stimulation method.

a) *Dog.* In 48 experiments there was found consistently on each side a nerve which supplies the inferior constrictor of the pharynx and the entire cervical portion of the esophagus. This nerve is about one third of the size of the superior laryngeal nerve and is found dorsal and almost at a right angle to the latter at a level just above the upper border of the thyroid cartilage. It goes posteriorly and medially to lie on the dorsolateral aspect of the lower part of the pharynx. Then it courses posteriorly, in a slightly zigzag way, toward the lateral aspect of the esophagus, where it breaks up into several fine branches. Along its course after crossing the superior laryngeal nerve it was found to give a communicating branch to the external branch of the latter in about one half of the experiments. Stimulation of the external branch of the superior laryngeal after it receives this communicating branch to the external branch gives rise to a weak or moderate tetanic contraction of the cervical portion of the esophagus. This explains the result obtained in one of the preliminary experiments described before. Occasionally this nerve breaks up just above or a little below the superior laryngeal nerve into two branches, medial and lateral, which reunite before it goes to the esophagus. Fine branches are given to the inferior constrictor muscle of the pharynx from the nerve trunk or from its medial branch when it is present. When it is traced cephalad, it goes laterally and slightly dorsally, crossing the ventral surface or the lower border of the superior cervical sympathetic ganglion, where in the great majority of cases strong fibrous tissues bind it closely to the ganglion and also to a small nerve given off by the ganglion to the carotid sinus. Then it soon joins the superior pharyngeal nerve to form a short common trunk which enters into the vagus nerve just above the nodose ganglion. It is hard to make sure by gross dissection whether the sympathetic ganglion gives fibers to this nerve or not, but stimulation of the ganglion gave no apparent change of the esophagus. Occasionally this nerve has no connection with the branch of the sympathetic ganglion at all, or it may break up into two branches, of which only one comes into relation with that of the sympathetic. They unite as usual before they go to the esophagus.

As a general rule this nerve can be easily identified as a small nerve lying on the dorsolateral aspect of the lower part of the pharynx slightly dorsal to the external branch of the superior laryngeal nerve. It is present on both sides in all experiments except one, in which this nerve was not found on the left side but was present normally on the right.

According to its physiological action so far ascertained it seems appropriate to designate this nerve as the pharyngo-esophageal nerve.

The effect of the recurrent laryngeal nerve on the cervical portion of the esophagus as determined by stimulation of the peripheral end of the vagus or the recurrent laryngeal nerve at the base of the neck is rather variable. In the majority of the

experiments there was contraction of the lower fourth or third of the cervical portion of the esophagus while the upper part was not affected. Stimulation of the pharyngo-esophageal nerve in these cases usually gave rise to contraction of the whole cervical portion, which is about one third of the entire length of the esophagus. However, in about one fifth of the experiments stimulation of the vagus caused contraction of the esophagus including the lower two thirds or even the entire length of the cervical portion. In these cases the pharyngo-esophageal nerve may cause contraction of only the upper two thirds of the cervical portion on stimulation.

b) *Cat.* In 14 experiments the cervical portion of the esophagus received its motor innervation almost solely from the pharyngo-esophageal nerve. It arises from the vagus nerve just above the nodose ganglion in a short common trunk with the superior pharyngeal nerve. It goes medially towards the pharynx, being almost parallel but dorsal and anterior to the superior laryngeal nerve. As soon as it reaches the pharynx at its dorsolateral aspect it gives branches to the inferior constrictor of the pharynx; then it courses posteriorly dorsal to the terminal branches of the superior laryngeal nerve to lie on the lateral surface of the esophagus where it branches freely. Stimulation of this nerve resulted in immediate tetanic contraction of the cervical portion, which, similar to the dog, is about one third of the entire length of the esophagus.

One interesting variation may be recorded here. It was found in two animals that there was a communicating branch between the superior laryngeal and the pharyngo-esophageal nerves. Results obtained by stimulation of the different portions of these nerves indicated that some motor fibers for the esophagus join the pharyngo-esophageal nerve by way of the superior laryngeal and the communicating branch. Stimulation of the superior laryngeal distal to the communicating branch had no effect on the esophagus—a condition different from that described in the dog.

The effect of stimulation of the peripheral end of the vagus nerve was confined to the thoracic portion of the esophagus. Only occasionally the lowermost inch or so of the cervical portion of the esophagus was involved. The effect of vagal stimulation on the thoracic esophagus was different from that in the dog. The upper thoracic part or the middle third of the esophagus responded by a strong, immediate and sustained contraction, while the lower third showed a sluggish spasmodic contraction of slow onset.

c) *Monkey (Macacus Rhesus).* The result of the experiments in three monkeys in regard to the innervation of the cervical portion of the esophagus was entirely in accord with Kahn's report. The external branch of the superior laryngeal nerve possesses an esophageal branch, stimulation of which caused strong contraction of the upper three fourths of the cervical portion of the esophagus. The cervical portion of the esophagus in this species of animal is about one fifth of the entire gullet in length. Stimulation of the pharyngeal branch of the vagus caused contraction of the pharyngeal muscles, the esophagus not being involved. Stimulation of the peripheral end of the vagus caused contraction of the entire length of the esophagus. It is interesting to record here that in response to vagal stimulation the middle third of the esophagus showed an immediate tetanic contraction followed after a period of short latency by further, but sluggish, contraction. This latter occurred simultaneously with, and similar in character to, the contraction of the lower third during

the stimulation.   Apparently these findings could be explained on the basis of the known muscular constituents of the esophagus of this species.

d) *Rabbit*.   In the first two experiments it was found that stimulation of the superior laryngeal nerve caused no contraction of the esophagus.   No nerve was present in the same anatomical position as the pharyngo-esophageal nerve in the dog or cat.   Stimulation of the peripheral end of the vagus in the neck caused strong contraction of the entire length of the esophagus.   This was in accord with all the previous reports (15, 16b, 17) and there has been no controversy in this species of animal.   In his experiments in the rabbit, Reid (15) stated that: "When the vagi are cut in the neck, even as high as the origin of the superior laryngeals, the pharynx and a very small portion of the esophagus next to it, still retain their healthy action, for, as we have already seen, these receive their motor filaments from the pharyngeal branches of this nerve."   However, apparently he based this statement upon his observation in the dog and in neither case did he dissect out the special branch to the esophagus.

In later experiments we found that on stimulation of the central end of the vagus nerve a reflex contraction of the upper end of the esophagus was obtained, and was not affected after section of the other vagus nerve in the neck and section of the superior laryngeal nerve on both sides.   Judging from the results of the study of the reflex contraction of the esophagus on stimulation of the central end of the vagus in the dog, which will soon be described in this communication, we were led to look for some additional nerve supply to the upper end of the esophagus other than the vagus.   On careful dissection an esophageal branch was found given off from the pharyngeal branch of the vagus but was hidden underneath the pharyngeal muscles on both sides.   Stimulation of this branch caused contraction of the upper half of the cervical portion of the esophagus, while section of this branch homolateral to the vagus being stimulated at the central end greatly diminished the reflex response of the esophagus, and subsequent section of the same branch on the other side abolished the reflex esophageal contraction completely.   Similar results were obtained in three rabbits.

e) *Guinea pig and albino rat*.   In both of these species the upper half of the cervical portion of the esophagus receives its innervation mainly from the external branch of the superior laryngeal nerve, as evidenced by its marked contraction on stimulation of the nerve in three animals of each species.   Stimulation of the peripheral end of the vagus caused contraction of the entire length of the esophagus but that of the upper third of the cervical portion was much weaker.

Thus, it is seen that a separate pharyngo-esophageal nerve was found in the dog and cat and a special esophageal branch in the other species of animals studied. Judging from the effect on the cervical portion of the esophagus on faradic stimulation, there seems to be little doubt about their physiological action, which, however, is further supported by the following experiments in the dog and cat.

2. *Experimental Evidences That the Pharyngo-esophageal Nerve is the Main Motor Nerve Supply to the Cervical Portion of the Esophagus*

a) *Acute experiments*.   The effect of faradic stimulation of the pharyngo-esophageal nerve on the cervical portion of the esophagus has been described above.

Stimulation of this nerve and the peripheral end of the vagus at the same time always gave a strong tetanic contraction of the entire length of the esophagus. When the esophagus including the recurrent laryngeal nerves was transected at the base of the neck and the cervical portion was then freed from all the adjacent connections, stimulation of the pharyngo-esophageal nerve demonstrated very clearly the simultaneous contraction of the longitudinal and circular muscular layers, as shown by the simultaneous shortening and constriction of the tube.

In some experiments in the dog, when a balloon inflated with about 20 cc. of air was placed in the esophagus at the junction of the cervical and thoracic portions, it could be pushed up into the cervical portion by stimulation of the peripheral end of the vagus nerve or down into the thoracic portion by stimulation of the pharyngo-esophageal nerve, evidently due to the strong tetanic contraction of the different portions of the tube.

When the cervical portion of the esophagus was freely exposed and the central end of the superior laryngeal nerve stimulated under light ether anesthesia, reflex swallowing was easily induced in the dog and followed by a distinct peristaltic wave. The wave passing along the thoracic portion could be felt by putting a finger into the lower esophagus through gastrostomy. The effect of sectioning the vagi in the middle of the neck or the pharyngo-esophageal nerves on the peristaltic movements of the esophagus was thus easily determined. Six experiments were done and results were consistent. Bilateral vagotomy did not affect the peristalsis over the cervical portion of the esophagus but abolished that over the thoracic. Subsequent section of the pharyngo-esophageal nerve on both sides made the whole tube quiescent. When this nerve was cut on both sides before the vagi, there was no more peristalsis over the cervical but still over the thoracic portion of the esophagus. Unilateral section of the nerve has no apparent effect on the peristalsis.

b) *Chronic experiments.* In order to ascertain the rôle of the pharyngo-esophageal nerve in the normal animal, this nerve was cut aseptically on both sides just above the level of the cricoid cartilage under local or general anesthesia in two dogs and three cats. In all animals similar symptoms of dysphagia and regurgitation developed. In some animals coughing occurred very often during a meal.

By roentgenological examination with a barium meal in the dog, paralysis of the cervical portion of the esophagus with retention of the meal was revealed, although the thoracic portion of the esophagus was functionally normal. Balloon examinations also checked very well with this finding (fig. 1c). Operation in the same anatomical region but cutting the superior laryngeal nerve instead of the pharyngo-esophageal resulted in no apparent disturbance of deglutition.

Roentgenological examination in the cat showed that the paralysis involved the whole cervical portion as well as the adjacent upper fourth of the thoracic portion of the esophagus (fig. 1d), while the portion below was active.

In the course of from three to five weeks each animal with the pharyngo-esophageal nerve sectioned became free from all these symptoms and roentgenological examination was negative except in the dog, which still showed slight retention of meal in the upper end of the cervical portion of the esophagus two and a half months or longer after the operation (fig. 1g).

In order to further evaluate the function of the pharyngo-esophageal nerve in the cat, one experiment was done in which the vagus nerves were sectioned aseptically
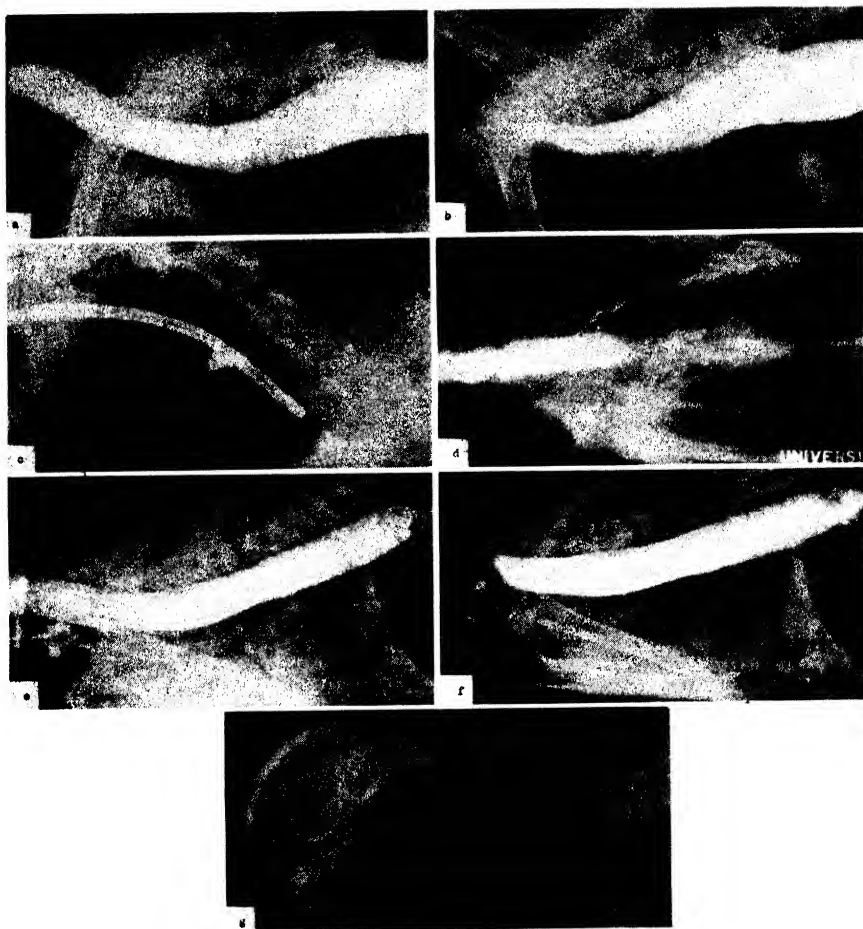
Fig. 1a. ROENTGENOGRAPH showing the condition of the esophagus in a dog after bilateral vagotomy at the level just posterior to the larynx. Milk containing barium sulfate has been given to the animal. b. Showing the same animal as in a. The roentgenograph was taken during deglutition. The peristaltic function of the cervical portion of the esophagus was preserved but the part below was paralyzed. c. Retention of a balloon in the paralyzed cervical portion of the esophagus after bilateral section of the pharyngo-esophageal nerve in a dog. d. Retention of barium meal in the cervical portion and the upper fourth of the thoracic portion of the esophagus after bilateral section of the pharyngo-esophageal nerve in a cat. e. Appearance of the esophagus after a barium meal in a cat after bilateral vagotomy in the neck posterior to the level of the larynx. Tracheotomy was also performed. f. Roentgenograph taken during deglutition of the same cat as shown in e. The cervical portion of the esophagus still possessed peristaltic activity. g. Retention of barium meal in the upper third of the cervical portion of the esophagus in a dog two and a half months after bilateral section of the pharyngo-esophageal nerve.

in the neck posterior to the level of the larynx together with tracheotomy under local anesthesia. Subsequent barium meal examinations revealed peristaltic function confined to the cervical portion of the esophagus (fig. 1e, f). Paralysis of the thoracic

portion of the esophagus was complete and no tertiary peristalsis appeared in a period of 10 hours after the operation.

3. *Reflex Spasmodic Contraction of the Esophagus on Stimulation of the Central End of the Vagus Nerve*

Meltzer and Auer (35) reported that a reflex tetanic contraction of the esophagus on stimulation of the central end of the vagus nerve is quite a reliable finding in the dog. When weak stimulation is used the tetanic contraction is confined to the cervical portion of the esophagus, but with a much stronger stimulation the thoracic portion also may be involved. They did not study the efferent pathway involved in this reflex, and no confirmation of their work was reported.

In the course of the present investigation with light ether or nembutal anesthesia we demonstrated in dogs consistently that there was a reflex tetanic contraction of the lower part of the pharynx and the cervical portion of the esophagus on stimulation of the central end of the vagus nerve. There was usually a period of latency of from two to four seconds, and the onset of the tetanic contraction was often preceded by some irregular twitchings. As soon as the stimulation stopped there was immediate relaxation. Subsequent stimulation of the same nerve gave the same response, but the period of latency was often much reduced.

It was consistently found that section of the vagus of the opposite side has no effect on this reflex. Except in one case this reflex was always abolished by section of the pharyngo-esophageal nerve of the same side. In these cases stimulation of the central end of the vagus nerve of the opposite side again produced the same phenomenon but also was abolished by section of the pharyngo-esophageal nerve of that side. In the exceptional case the reflex was abolished only by bilateral section of the nerve.

4. *Periodic Spasm of the Esophagus During Gasping*

In one dog under ether anesthesia after the chest was opened with artificial respiration, gasping type of respiration developed during the course of the experiment without apparent reason. At the same time there was a periodic spasmodic contraction of the cervical portion of the esophagus corresponding to the later part of the inspiratory phase of the gasping. This spasmodic contraction disappeared after the spontaneous cessation of the gasping.

In subsequent experiments efforts were made to reproduce the same phenomena. Gasping type of respiration was regularly produced in 8 dogs several minutes after ligation of the common carotid and the vertebral arteries on both sides at the base of the neck and was always associated with the periodic esophageal spasm during the later part of the inspiration. The strength of the spasm apparently correlated with the degree of the gasping.

In one experiment spasm of the esophagus thus produced was found involving the entire length of the esophagus, as determined by putting a finger into the lower thoracic esophagus through the cardia. In another one the same thing was found by direct observation after the chest was opened. In the rest of the experiments, whether or not the thoracic portion of the esophagus was involved was not determined.

In the experiments described above section of both vagus nerves in the neck

abolished the spasm of the entire thoracic portion of the esophagus but had no effect on that of the cervical portion. The latter was abolished by bilateral section of the pharyngo-esophageal nerve in all the experiments.

DISCUSSION

Review of the literature indicates that the interesting reports of the earlier workers (16–18) concerning the innervation of the cervical portion of the esophagus have been entirely ignored by the later investigators. As a matter of fact these old but fundamental conceptions regarding the neuromuscular mechanism of this organ are not taught to students of physiology, who, on the other hand, are always impressed by the traditional teaching that this part of the organ is controlled by the recurrent laryngeal branch of the vagus nerve regardless of the species. This classical conception is apparently based mainly on the experiments in the rabbit, which have given consistent results probably ever since the time of Reid (15), and has been generalized to all the mammals, among which the dog and the cat have been the commonly used experimental animals. Not only the recent workers (22–27, 31) have supported the present conception, but also the earlier investigator, Meltzer, who made brilliant studies on the peristaltic activities of the esophagus, apparently had the same idea. During his experiments of transecting the esophagus in the dog (36, p. 268), he emphasized that the recurrent laryngeal nerves should not be injured, and mentioned no nerve supply from other sources.

The present investigation among the different species of the ordinarily used laboratory animals, however, strongly supports the overlooked old idea that the cervical portion of the esophagus does receive some motor innervation in addition to the recurrent. There is no exception even in the rabbit to the general finding that the cervical portion of the esophagus receives a double innervation from widely separated vagal branches, although there are species variations in regard to their anatomical course and extent of distribution.

Evidence has been presented in both acute and chronic experiments in the dog proving that the pharyngo-esophageal nerve is the motor nerve that controls all the following activities of the cervical portion of the esophagus. These activities include peristalsis, the reflex tetanic contraction on stimulation of the central end of the vagus nerve and the periodic spasm during gasping as observed in the acute experiments. Practically all these activities are abolished by bilateral section of this nerve. In chronic experiments in both the dog and cat, the cervical portion of the esophagus was not paralyzed by section of the vagi just posterior to the level of the larynx but by bilateral section of this pharyngo-esophageal nerve, although the permanent paralysis is limited only to the very upper portion in the dog. A diagram has been constructed to illustrate the motor innervation of the esophagus of the dog (fig. 2).

The question arises as to why such an important nerve has not drawn the due attention of recent investigators in this field. The old literature could have been overlooked, but the generalization of the manner of the innervation in the rabbit is not likely to be the only answer to the question of the origin of the current conception which, as pointed out in the review of the literature, has received much experimental

support purporting to prove that the cervical esophagus is innervated by the recurrent branch of the vagus.

There are several reasons that might account for the discrepancies. In the first place there could be such exceptional cases in the dog as noted in some of the present experiments, in which the entire cervical portion of the esophagus may receive motor innervation from the recurrent branch of the vagus in addition to the pharyngo-esophageal nerve. In such a case further efforts to search for an additional
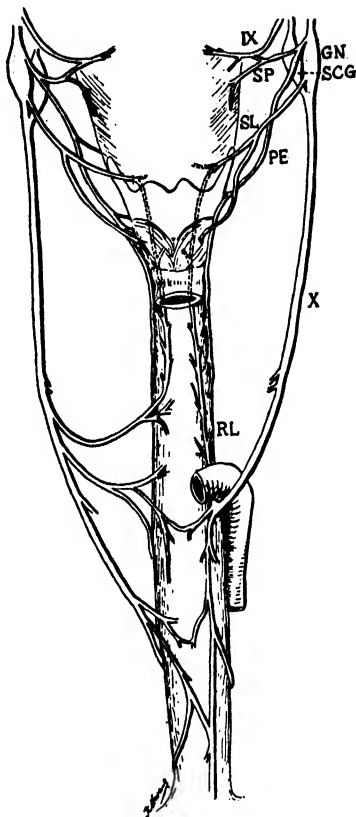
Fig. 2. DIAGRAM ILLUSTRATING THE INNERVATION of the esophagus in the dog. *GN:* ganglion nodosum; *PE:* pharyngo-esophageal nerve; *RL:* recurrent laryngeal nerve; *SCG:* superior cervical sympathetic ganglion; *SL:* superior laryngeal nerve; *SP:* superior pharyngeal nerve; *IX:* glossopharyngeal nerve; *X:* vagus nerve.

motor nerve are apparently not indicated. The manner of innervation of the esophagus in the rabbit is another good example.

In the second place, the method of the observation is very important in the interpretation of the results. Apparently several different methods have been used by these recent workers. By direct inspection Inaoka (22) observed that peripheral vagal stimulation does not act on the cervical esophagus, which is only pulled down passively. But direct stimulation of the recurrent nerve results in contraction of the cervical esophagus. The failure of the vagal stimulation, he believes, is due to a block at the inferior cervical ganglion. This is not in accord with our knowledge of anatomy and we did not record similar results. The results of stimulation of the

vagus stump and of the recurrent laryngeal usually correlated very well when care was taken to avoid the spread of the current.

According to some investigators (23, 27) records of contraction of the cervical esophagus upon vagal stimulation have been obtained by means of a recording tambour connected to a balloon placed in the cervical portion of the esophagus. In this case it is not impossible that a purely mechanical traction of the cervical portion of the esophagus by the shortening of the thoracic portion on vagal stimulation may cause a rise of the pressure in the balloon simulating contraction, or the contraction of the lower part of the cervical esophagus could be interpreted as contraction of the entire cervical portion.

In chronic experiments the roentgenoscopic method has often been used (1, 26). Unless the deglutition movement is carefully observed as shown in figure 1b, f, roentgenographs such as figure 1a, e, may give rise to the impression of paralysis of the entire esophagus. Those who make vagotomy in the neck usually leave the right recurrent laryngeal nerve intact (20, 27), believing that the innervation of the cervical portion of the esophagus is preserved in this way. Jurica (26) reported complete paralysis of the striated portion of the esophagus after he made successful double vagotomy at the middle of the neck in the cat. However, examination of the roentgenographs in his article reveals no retained meal in the cervical esophagus, a fact which could be due to the preserved motor innervation by the pharyngo-esophageal nerve as shown in the present experimentation.

One conception that might lead people to overlook the activity of the cervical portion of the esophagus is the effect of the squirting action by the rapid contraction of the muscles of the mouth. By this action liquid and semisolids are squirted down the esophagus instead by being moved by peristalsis. This is proposed by Kronecker and Meltzer (37) and supported by Cannon (38) and Meltzer (39). The latter demonstrated a dog drinking in a perfectly normal manner after the muscularis of the entire cervical esophagus has been removed. This is not in accord with the present report, for dysphagia, although temporary, did develop after cutting the pharyngo-esophageal nerves, and also retention of food (milk and barium sulfate) was found in the cervical portion of the esophagus (fig. 1g). This retained food detected by the roentgenologic method could not be explained simply by the separated barium sulfate stuck to the esophageal wall as suggested by Meltzer (39), since we have never succeeded in causing barium sulfate to stick to the esophageal wall of the normal dog by using different food mixtures, liquid or solid. The copious secretion of the esophageal glands of this animal may prevent adherence of the barium. Judging from these points we can say that the 'squirting' action is not the only mechanism by which liquid meals pass through the cervical portion of the esophagus and that for completing the process of deglutition, normal peristaltic activities of this portion of the organ cannot be neglected.

The immediate paralysis of the cervical portion of the esophagus following bilateral section of the pharyngo-esophageal nerve in the dog and cat signifies the important rôle played by this nerve in normal physiological function. The discrepancy that the length of the paralyzed portion of the esophagus following bilateral section of the pharyngo-esophageal nerve is longer than what is expected on the basis

of stimulation of this nerve in acute experiments in the cat is not readily understood. Although there is complete functional recovery in the course of from three to five weeks, residual signs in the dog could still be found two to four or more months later. This functional recovery of the once paralyzed organ cannot be explained by regeneration of the nerve since, on the one hand, a period of three or five weeks is too short for the possible regeneration, and on the other hand, the recovery is not complete at the uppermost portion in the dog.   Further investigation is being carried on to find the correct explanation, but in all probability the function is taken over by the recurrent laryngeal nerves.   This assumption is based upon the fact that the recovery is more complete over the lower part of the cervical portion of the esophagus, which is often found to be innervated by both the pharyngo-esophageal and the recurrent laryngeal nerves.   Should this be true, the significance of the double innervation of this organ is evident.

As pointed out before, the anatomical course of the pharyngo-esophageal nerve, as determined by dissection and electric stimulation in the dog and cat, is variable. In general it is well in accord with what is described by Espezel and Kahn.   Regarding its origin, we agree with Kahn, that is, it originates from the vagus in a common trunk with the superior pharyngeal nerve and not from the superior cervical sympathetic ganglion as claimed by Espezel.   However, its close relationship to this ganglion, as described before, is noticeable.   Probably the finding of stasis of food in the upper esophagus after extirpation of this ganglion in the dog, as reported by Kure and others (32, 33), could be explained on the basis of incidental resection of that portion of the nerve which is bound to the ganglion.

The mechanism of the periodic spasmodic contraction of the esophagus during gasping in the dog certainly deserves further investigation.   The efferent pathways are apparently the vagus nerve for the thoracic portion and the pharyngo-esophageal for the cervical.   Since this spasmodic contraction occurs in the inspiratory phase and its strength is usually correlated with the extent of the gasping, it seems rational to assume that the esophageal musculature, at least the thoracic portion, could be considered as one of the accessory respiratory muscles.

Regarding the innervation of the human esophagus little more is known than the classical conception.   Since there are wide variations among different mammals it is difficult to predict to which species the manner of innervation of the human esophagus might correspond.   No nerve corresponding to the pharyngo-esophageal has been described in the human being, nor any esophageal branches from the superior laryngeal.   However, clinical reports of 'cardiospasm' with paralysis of the lower two thirds of the esophagus (40) and 'dysphagia' due to paralysis of the upper third of the esophagus (41) should call our attention to the possibility of separate innervation of the different portions of the human esophagus.

SUMMARY

The innervation of the esophagus has been studied in the dog, cat, rabbit, monkey, guinea pig and rat.   There is no exception to the general conclusion that the cervical portion of the esophagus receives double innervation from widely separate vagal branches.   Species differences regarding the anatomical course and the extent

of distribution have been described and discrepancies of opinions among different authors according to the literature have been discussed.

The main motor innervation of the cervical portion of the esophagus in the cat and dog is the pharyngo-esophageal nerve, which arises from the vagus above the ganglion of nodosum. Stimulation of this nerve in these animals causes strong tetanic contraction of the cervical portion of the esophagus.

In chronic experiments in the dog and cat, bilateral section of the vagus trunk alone resulted in paralysis of the whole esophagus except the cervical portion. After bilateral section of the pharyngo-esophageal nerve alone there was temporary paralysis of the entire cervical esophagus accompanied by symptoms of dysphagia. Apparent functional recovery was complete in three to five weeks.

In acute experiments in the dog, that the pharyngo-esophageal nerve is the main efferent nerve to the cervical portion of the esophagus is further evidenced by the abolition of all of its activities after section of this nerve. The activities of this portion of the esophagus here include the peristaltic movements, the reflex tetanic contraction on stimulation of the central end of the vagus nerve, and the periodic spasm during gasping.

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EFFECT OF SECRETIN AND PANCREOZYMIN ON AMYLASE AND ALKALINE PHOSPHATASE SECRETION BY THE PANCREAS IN DOGS

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TWO hormones extractable from the upper intestinal mucosa, secretin and pancreozymin, are concerned in the regulation of pancreatic secretion. This study was undertaken in order to obtain further information on the rôle of each of these hormones in the control of enzyme secretion by the pancreas.

Previous work has shown that secretin acts as a stimulus mainly for water and bicarbonate secretion by the pancreas. Thus, the pancreatic juice secreted in response to it is relatively poor in enzymes when compared with either pancreozymin plus secretin stimulation or stimulation by parasympathomimetic drugs. By contrast, pancreozymin (10–12) stimulates the secretion of enzymes (amylase, lipase and trypsinogen) without influencing the rate of water secretion, that is without changing the volume of juice put out per minute.

While it is apparent that secretin does not evoke a juice rich in enzymes, there are discrepancies in the literature concerning whether it has any stimulating action on enzyme secretion (1–9). Some of these discrepancies can probably be explained by the presence of varying amounts of pancreozymin in the secretin preparations that were tested. Therefore, the first question which we set out to answer was, does an increase in the amount of circulating secretin increase significantly the total output of amylase in the pancreatic juice per unit of time?

Another question that it was considered desirable to investigate pertained to the secretion of alkaline phosphatase in the pancreatic juice of the dog (13,14). Histochemically phosphatase occurs only in the cells lining the ductules and is absent from the acinar cells (15–17). This is illustrated in figure 1, a photomicrograph of a section of dog's pancreas stained by the Gomori technique for alkaline phosphatase (15). The effect of secretin and pancreozymin on the secretion of alkaline phosphatase has not been studied previously.

METHODS

Acute experiments were carried out on dogs which were anesthetized with an intravenous injection of pentobarbital (32 mg/kg. body weight). Prolonged anesthesia was maintained when necessary by additional intravenous injections. The last feeding of the animals was 24 hours before the experiment. Cannulae were inserted into the left femoral vein for secretin infusion and also into the trachea.

To preclude stimulation of the pancreas by bile or acid in the duodenum, the common bile duct and the pylorus were occluded. Pancreatic juice was collected by a cannula inserted into the major pancreatic duct.

Highly purified secretin dissolved in saline was continuously injected intravenously by means of a perfusion pump into the left femoral vein of the animal. Three different secretin preparations were used. All were devoid of vasodepressor substances. Secretin *A* was about twenty times as potent as SI (11); secretin *B*, twelve times; and secretin *C*, eight times[1]. The pancreozymin used in the present work was prepared by the aniline precipitation procedure (11) and also showed no vasodepressor effect.

Fig. 1. PHOTOMICROGRAPH OF DOG'S PAN-CREAS stained for alkaline phosphatase by the method of Gomori. Only the ductule cells stain positively.

The first injection rate was adjusted so as to maintain a rate of flow of juice of about 1.5 to 3.0 cc. in 15 to 30 minutes. Increase of flow was brought about by increasing the rate of injection of secretin. In order to clear out the initial concentrated juice accumulated in the pancreatic duct system, in the cannula and in the connecting tube and, also, owing to the fact that animals were usually more sensitive to the secretin stimulation in the beginning, the juice collected during the first hour of response to secretin was discarded. Pancreozymin was injected in a single dose of 20 mg. of the preparation employed during the response to a constant dose of secretin. The pancreatic juice was collected in 15 to 30 minute portions, in graduated centrifuge tubes on ice, and was stored in the freezing unit of the refrigerator overnight. At least three or four samples were collected at each secretory rate level. All samples were assayed for amylase and alkaline phosphatase content.

The amylase content of successive samples of juice was measured by the modified Willstatter method described in detail by Schmidt, Greengard and Ivy (18). The amylolytic activity unit was expressed in mg. of maltose per cc. of juice as derived from the thiosulphate titration. From the amylase content of the various samples, the minute output of this enzyme was calculated by multiplying the concentration in mg/cc. by the volume of the sample in cc. divided by the duration of the collection in minutes.

[1] These secretin preparations were kindly supplied by Dr. E. D. Campbell of the Lilly Research Laboratories, Indianapolis, Ind.

According to the generally accepted conception that the three principal enzymes of the pancreatic juice, namely the amylase, trypsinogen and lipase, are parallel in their concentration (9, 10, 11, 20, 21), reliance has been placed on the estimation of amylase as an index of the concentration changes of the three principal enzymes of the juice under the experimental conditions. The method for amylase determination was simpler and gave much greater sensitivity than could be obtained for the other two enzymes.

The content of alkaline phosphatase of the juice was estimated essentially by the method described by Shinowara, Jones and Reinhart (19). The final pH of the reaction mixture of substrate and pancreatic juice is 9.3 ± 0.15 at $37°$ C. The method for the estimation of the 'initial' inorganic phosphate of juice and of the 'total' inorganic phosphate of juice after incubation was devised by Dr. J. Canepa of this laboratory after Holman's method for inorganic phosphate. The latter method is outlined briefly as follows: To 1 cc. of 'total' phosphate protein-free fluid, in a 15 cc. graduated conical centrifuge tube, water is added to make the total volume 5 cc. At the same time a 'blank' is prepared by using 1 cc. of 'blank fluid' instead of 'total' phosphate protein-free fluid. 'Blank fluid' is prepared similarly to the 'total' phosphate protein-free fluid by using water instead of pancreatic juice before incubation. To all the tubes add in succession 1 cc. of 10 N H_2SO_4, 1 cc. of 2.5 per cent ammonium molybdate and 1 cc. of 20 per cent KI (containing 0.5 per cent Na_2CO_3). Mix by tapping after each addition. Put into a boiling water-bath for exactly 15 minutes. Cool. Next add 0.4 cc. of 0.25 per cent anhydrous sodium sulphite. Bring the final volume to 10 cc. or 15 cc. depending upon the depth of the color.

The density of the color developed by the reduction of phosphomolybdic acid is read on a Coleman spectrophotometer at 670 mμ. From the readings, the amount of 'total' phosphate can be estimated by reference to a calibration curve constructed by using solutions containing known amounts of phosphate. The 'initial' inorganic phosphate of pancreatic juice is determined in the same way by using an 'initial' protein-free fluid instead of 'total' phosphate protein-free fluid. A 'blank' is prepared similarly to the preparation of 'initial' protein-free fluid by using water instead of pancreatic juice.

The 'total' inorganic phosphate in mg. of P per 100 cc. of juice after incubation minus the 'initial' inorganic phosphate in mg. of P per 100 cc. of juice without incubation equals the liberated inorganic phosphate. The alkaline phosphatase activity unit is therefore expressed in mg. of phosphorus per 100 cc. of pancreatic juice liberated from the incubation.

From the alkaline phosphatase contents of the various samples, the minute output of this enzyme was calculated by multiplying the concentration by the volume of the sample in cc. divided first by 100 and again by the time of collection of the sample in minutes.

RESULTS

In order first to show the effect of constant administration of secretin on the amylase and alkaline phosphatase content of the pancreatic juice over an experimental period, control experiments were carried out (table 1). The volume, amylase concentration and alkaline phosphatase concentration of the pancreatic juice, during constant administration of secretin over a period of three hours, is shown in the experiment depicted in figure 2. This experiment, begun after an hour's previous secretin stimulation, clearly demonstrates the general tendency of changes of the juice.

The volume usually showed a gradual increase and finally a plateau was reached. The amylase content showed little fluctuations, but generally there was a tendency to decline slowly toward the last phase of the experiment. A clear fall of the con-

centration, however, was observed in the case of alkaline phosphatase. Calculations (table 1) show that the minute output of amylase remained essentially unchanged while that of the alkaline phosphatase shows a slowly diminishing rate.

As regards the sensitivity of different animals to the effect of secretin, considerable variation was observed. This sensitivity can be judged from the rate of spontaneous flow of juice before the application of secretin stimulation and also from the

TABLE 1.   FLUCTUATIONS IN VOLUME AND IN CONCENTRATION AND MINUTE OUTPUT OF ALKALINE
PHOSPHATASE AND AMYLASE OF PANCREATIC JUICE DURING CONSTANT
SECRETIN ADMINISTRATION (2 DOGS)

| | DOG NO. | PORTION NUMBER | | | | | | | | | | | |
		1	2	3	4	5	6	7	8	9	10	11	12
Volume (cc.)	2	3.2	3.1	3.3	3.6	3.5	4.1	4.0	3.7	3.9	4.0	3.9	4.0
	8	2.8	3.2	2.9	4.0	4.5	4.7	4.1	4.5	5.0	5.0	5.0	
Amylase conc.	2	652	412	360	400	360	340	320	310	359	292	274	295
	8	473	442	414	391	380	410	476	313	341	346	330	
Amylase min. output	2	104.1	63.9	59.4	76.5	63.0	69.7	64.0	57.4	70.1	58.4	52.2	59.0
	8	88.0	94.0	80.0	104.0	114.0	128.5	130.0	93.3	114.0	115.0	110.0	
Alk. phosphatase conc.	2	3.00	3.40	3.00	1.70	2.40	2.00	2.40	2.00	1.60	1.80	1.20	1.00
	8	2.10	1.45	1.40	1.50	1.40	0.95	0.75	0.80	0.70	0.85	0.65	
Alk. phosphatase min. output	2	0.0048	0.0053	0.0050	0.0031	0.0042	0.0041	0.0048	0.0037	0.0031	0.0036	0.0023	0.0020
	8	0.0034	0.0031	0.0026	0.0040	0.0042	0.0030	0.0021	.0024	0.0023	0.0028	0.0022	

Dog 2, 9 kg. Secretin *B*: 3.4 mg/20 min.; samples collected in 20-min. portions.
Dog 8, 10 kg. Secretin *A*: 1.2 mg/15 min.; samples collected in 15-min. portions.
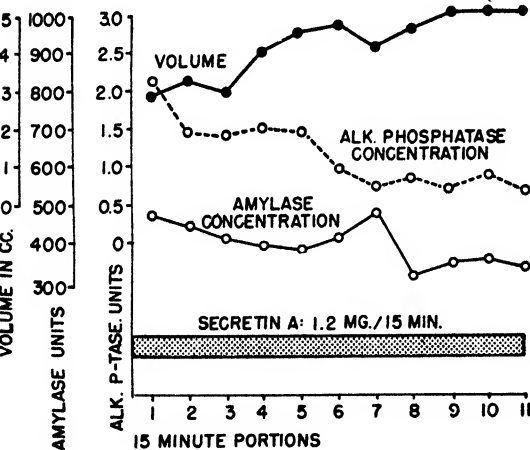
Fig. 2. *Dog 8*, 10 kg. Fluctuations in volume and in concentration of amylase and alkaline phosphatase of pancreatic juice during constant secretin administration

initial response of the gland to the first rate of secretin administration. Moreover, the sensitivity of the same animal to the effect of secretin is by no means constant. Generally speaking, they are more sensitive in the initial phase of secretin stimulation, then gradually become stabilized and maintain the condition for three or four hours or sometimes longer. It was for this reason that a previous secretin stimulation, of approximately one hour before the collection of samples, was necessary.

However, a phenomenon of exhaustion might be observed after an especially prolonged maximal stimulation over a period of one to two hours.   In both the experiments shown in table 1, a moderate intensity of secretin stimulation was maintained throughout the whole period of over three hours.   It was in this period that the most stable results were obtained.

The question as to whether or not secretin itself has any stimulating effect on the enzyme production of the pancreas has been answered in the results obtained from seven experiments.   The volume, enzyme concentration and enzyme minute output of the pancreatic juice obtained before and after the increase of secretin stimulus are shown in table 2.   Essentially similar results are obtained in different animals either with the secretin *A* or the secretin *B*.   Figure 3 drawn from the observations on *dog 12* serves as a typical illustration.   Portions 1 to 4 (inclusive) were taken as controls during a constant rate of perfusion of secretin.   The variation in the volume of juice was small.   The amylase concentration and minute output also showed only small fluctuations.   During the period represented by portions 5 to 7, the secretin stimulus was quadrupled, and the result was a sharp rise in volume soon reaching a plateau; concomitantly a great fall in amylase concentration occurred.   The minute output of the enzyme, however, remained essentially unchanged.   Although portion 5 did show an 8 per cent increase as compared with the previous one (portion 4), it could hardly be attributed to an increase of enzyme secretion under stimulation by secretin since as much as 38 per cent fluctuation of enzyme output occurred among the control portions 1 to 4.   Moreover, this rather insignificant increase of the enzyme output might be attributed to a 'washing out' from the gland of the enzyme which was presumably accumulated in the glandular passages of acini which were inactive before the increase of secretin stimulation took effect, the process therefore being a passive one.   This 'washing out' assumption is strongly supported by the evidence that the amount of the enzyme discharged in the portions 6 and 7, though still under an increased rate of secretin injection, did not show a continued increase following the immediate increase in portion 5.

In the experiment as represented in figure 3, the initial level of secretin injection rate (portions 1–4) was very low and the secretin stimulation was presumably not much above threshold, whereas in the second phase represented in portions 5 to 7 the secretin stimulus was definitely maximal.   The fact that secretin has no stimulating effect on enzyme production of the pancreas is quite evident.   Nor could this be attributed to exhaustion of the gland, since in some experiments, pancreozymin injected at the last phase of maximal secretin stimulation has never failed to cause an abundant increase in amylase concentration in the juice.

In order to demonstrate the effect of secretin on the alkaline phosphatase concentration and its minute output in the dog's pancreatic juice the same procedure has been used as with amylase.   The volume, alkaline phosphatase content and minute output of the juice obtained before and after the increase of secretin stimulus in five experiments are shown in table 3.   The observation from *dog 3* is illustrated in figure 4.   Portions 1 to 4 (inclusive) were collected under constant secretin stimulation (3.4 mg/20 min.).   For portions 5 and 6 the secretin stimulus was almost doubled and this resulted in a sharp rise in volume and a sharp fall in alkaline phosphatase

concentration. A more striking effect was observed by further increase of secretin injection in portions 7 and 8 (8 mg/20 min.). As can be seen from figure 4, the minute output of alkaline phosphatase showed a fall. This continued fall in alkaline

TABLE 2. EFFECT OF SECRETIN ON CONCENTRATION AND MINUTE OUTPUT OF AMYLASE (7 DOGS)

	DOG NO.	PORTION NUMBER									
		1	2	3	4		5	6	7	8	9
Volume (cc.)	3	2.8	3.7	4.2	4.5		7.4	8.2	9.2¹	9.8¹	
	4		2.1	3.0	4.3		6.8	6.5	6.8	7.0¹	7.5¹
	8	4.5	5.0	5.0	5.0		6.9	6.5	6.9		
	10	2.6	3.5	4.1	4.1	Increase of Secretin Injection Rate (see below)	12.9	14.8	14.0		
	11	0.9	0.9	1.6	1.9		4.1	4.6	5.5		
	12	1.5	1.4	1.4	2.3		6.1	8.7	8.8		
	13		3.1	3.0	4.3		8.0	8.4	8.8		
Amylase concentration	3	391	349	402	326		217	178	180¹	158¹	
	4		770	352	250		190	157	162	148¹	130¹
	8	313	341	346	330		138	199	152		
	10	1110	735	638	582		243	200	187		
	11	1261	1037	867	604		348	221	230		
	12	939	905	1118	761		310	200	174		
	13		417	540	510		319	270	205		
Amylase min. output	3	54.8	64.6	84.3	73.4		80.2	73.4	83.3¹	77.2¹	
	4		53.9	35.2	35.6		42.8	34.0	36.4	34.5¹	35.0¹
	8	93.3	114.0	115.0	111.0		64.0	86.0	70.0		
	10	192.2	171.5	174.4	159.1		209.0	197.0	173.6		
	11	45.4	33.3	55.5	45.9		54.5	40.7	50.6		
	12	70.5	63.4	78.3	87.5		95.0	86.9	74.7		
	13		86.2	108.0	146.2		170.0	151.2	120.1		

Dog 3, 9.7 kg. Secretin *B*: 3.4 mg/20 min. during portions 1–4; 6 mg/20 min. during portions 5 & 6; 8 mg/20 min. during portions 7 & 8; samples collected in 20-min. portions.

Dog 4, 11 kg. Secretin *B*: 6 mg/30 min. during portions 2–4; 12 mg/30 min. during portions 5–7; 15.2 mg/30 min. during portions 8 & 9; samples in 30-min. portions.

Dog 8, 10 kg. Secretin *A*: 1.2 mg/15 min. during portions 1–4; 4.5 mg/15 min. during portions 5–7; samples in 15-min. portions.

Dog 10, 15.5 kg. Secretin *A*: 0.65 mg/15 min. during portions 1–4; 2.7 mg/15 min. during portions 5–7; samples in 15-min. portions.

Dog 11, 14.2 kg. Secretin *A*: 1.1 mg/25 min. during portions 1–4; 4.5 mg/25 min. during portions 5–7; samples in 25-min. portions.

Dog 12, 8 kg. Secretin *A*: 0.33 mg/20 min. during portions 1–4; 1.47 mg/20 min. during portions 5–7; samples in 20-min. portions.

Dog 13, 9.5 kg. Secretin *A*: 0.313 mg/15 min. during portions 2–4; 1.38 mg/15 min. during portions 5–7; samples in 15-min. portions.

¹ Second increase of the rate of secretin injection (see above).

phosphatase output is characteristic of such secretin injection experiments, both those in which a constant lower rate of injection is used throughout the whole experiment (table 1) as well as those in which variations in the rate of the secretin adminis-

Fig. 3. *Dog 12*, 8.0 kg. Effect of secretin on concentration and minute output of amylase. Increase of the rate of secretin injection started after collecting portion 4.
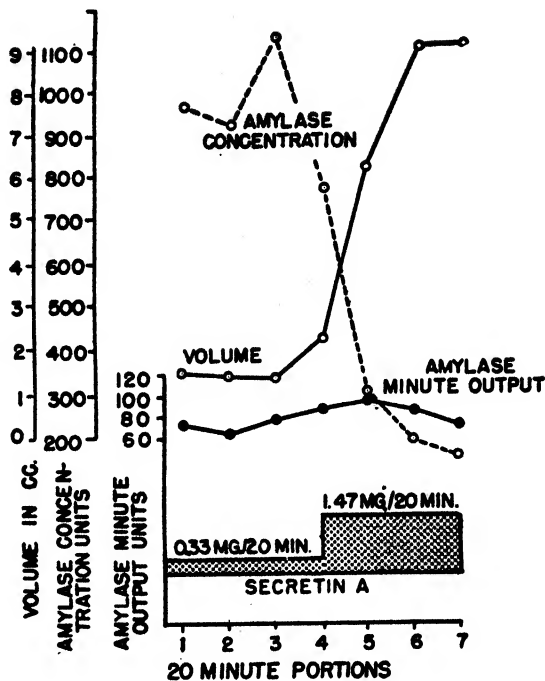
TABLE 3. EFFECT OF SECRETIN ON CONCENTRATION AND MINUTE OUTPUT OF ALKALINE PHOSPHATASE (5 DOGS)

	DOG NO.	PORTION NUMBER								
		1	2	3	4	5	6	7	8	9
Volume (cc.)	3	2.8	3.7	4.2	4.5	7.4	8.2	9.2¹	9.8¹	
	4		2.1	3.0	4.3	6.8	6.5	6.8	7.0¹	7.5¹
	8a		2.4	2.4	2.6	3.6	3.6	4.0	4.0	3.6
	8	4.5	5.0	5.0	5.0	6.9	6.5	6.9		
	10	2.6	3.5	4.0	4.1	12.9	14.8	14.0		
Alkaline phosphatase concentration	3	7.90	5.50	4.40	4.70	2.23	1.40	1.30¹	0.85¹	
	4		6.20	2.90	2.40	2.50	2.20	1.60	1.20¹	1.40¹
	8a		0.52	1.15	0.89	0.75	0.37	0.37	0.52	0.32
	8	0.80	0.70	0.85	0.65	0.45	0.40	0.35		
	10	18.25	12.10	9.65	7.95	4.24	3.20	3.55		
Alkaline phosphatase min. output	3	0.0111	0.0102	0.0092	0.0101	0.0083	0.0057	0.0061¹	0.0042¹	
	4		0.0043	0.0029	0.0034	0.0056	0.0050	0.0036	0.0028¹	0.0035¹
	8a		0.0012	0.0028	0.0023	0.0027	0.0013	0.0015	0.0021	0.0011
	8	0.0024	0.0023	0.0028	0.0022	0.0021	0.0017	0.0016		
	10	0.0316	0.0282	0.0264	0.0217	0.0360	0.0315	0.0330		

Note: the vertical label between columns 4 and 5 reads "Increase of Secretin Injection Rate".

Dog 8a, 9 kg. Secretin C: 2.38 mg/10 min. during portions 2–4; 4.75 mg/10 min. during portions 5–9; samples in 10-min. portions.

For *dog 3, 4, 8 & 10*, see the remarks in table 2.

[1] Second increase of the rate of secretin injection (see above).

tration occur (fig. 4).   The fact that secretin does not seem to stimulate the production of alkaline phosphatase in the pancreas was clearly shown.   This continued reduction of alkaline phosphatase output following the prolonged administration of secretin might be explained by assuming that the output was not balanced by synthesis.

The results of the experiments with pancreozymin are shown in table 4.   All the four experiments were carried out under constant stimulation with secretin. Figure 5 plotted from the results observed on *dog 6* shows the typical changes.   As can be seen from the first phase of figure 5 (portions 1–4), both the volume and the amylase concentration showed a constant level.   The concentration of alkaline phosphatase, however, fluctuated a little and showed the usual fall.   Pancreozymin was injected in a single dose of 20 mg. in the beginning of the second phase (portions
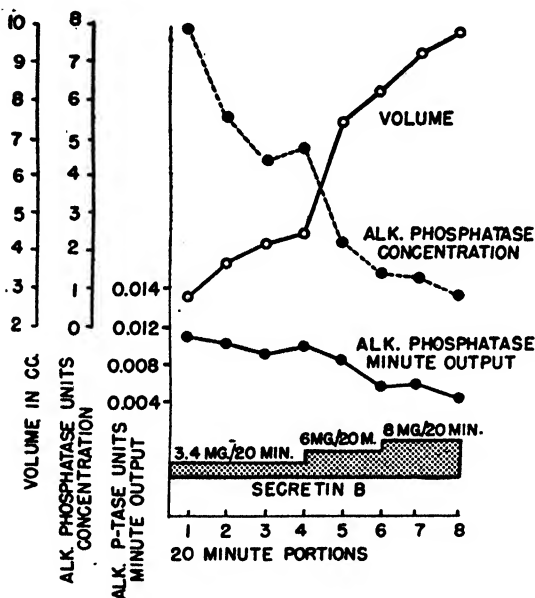
Fig. 4. *Dog 3*, 9.7 kg.   Effect of secretin on concentration and minute output of alkaline phosphatase.   First increase of the rate of secretin injection started after collecting portion 4. Second increase started after portion 6.

5–8) after collecting portion 4 and again injected in a single dose of 14 mg. in the third phase (portions 9–12) after collecting portion 8.   There resulted a three-fold increase of amylase concentration in portion 5 and a more than two-fold increase in portion 9.   The concentration of alkaline phosphatase showed a slight decrease. This preparation of pancreozymin did have a slight secretin activity as shown in the rate of flow of juice at portions 5 and 9 in figure 5.   However, this slight secretin activity had no confusing effect in these experiments, since the fact that secretin itself has no effect on either amylase or alkaline phosphatase concentration has been repeatedly shown in the experiments already presented.

As can be seen from table 4, the minute output of alkaline phosphatase showed a slowly diminishing fall comparable to that of table 1, in which pancreozymin was not injected.   Apparently, pancreozymin has no effect whatever on alkaline phosphatase secretion by the pancreas.

TABLE 4.—EFFECT OF PANCREOZYMIN ON AMYLASE AND ALKALINE PHOSPHATASE (4 DOGS)

	DOG NO.	PORTION NUMBER											
		1	2	3	4	5	6	7	8	9	10	11	12
Volume (cc.)	6	4.0	3.9	4.0	3.9	5.3	3.2	2.6	2.8¹	3.5	2.3	3.4	3.6
	7		6.6	7.3	7.0	9.3	7.3	6.8¹	8.7	7.0	6.2		
	8	4.6	4.0	3.6	3.6	3.1	2.4	2.4	3.4	3.0			
	9		4.0	4.0	3.5	4.5	3.9	3.0	3.4	3.0			
Amylase conc.	6	277.8	285.3	291.9	282.5	880.4	280.6	301.3	442.6¹	748.6	541.4	337.1	290.0
	7		890.2	883.5	876.1	1163.4	866.6	860.0¹	1107.0	965.6	902.4		
	8	152.0	163.0	167.0	148.0	853.0	319.0	226.0					
	9			790.0	790.0	2258.0	900.0	750.0	650.0	790.0			
Amylase min. output	6	111.1	111.2	116.8	110.2	466.6	89.8	78.3	113.9¹	262.0	114.5	114.6	104.4
	7		587.5	645.0	613.3	1082.0	632.0	584.8¹	963.1	675.9	559.5		
	8	70.0	65.2	60.0	53.3	264.4	76.5	54.2					
	9			316.0	276.5	1016.4	351.0	225.0	221.0	237.0			
Alkaline phosphatase conc.	6	1.46	1.78	1.47	1.15	0.81	1.25	1.67	1.67¹	1.20	1.67	0.83	0.94
	7		3.78	3.36	3.47	2.31	3.15	3.36¹	2.94	3.05	3.57		
	8	0.35	0.50	0.35	0.35	0.35	0.30	0.45					
	9		1.90	1.55	1.55	1.35	1.50	1.95	1.30	1.30			
Alkaline phosphatase min. output	6	0.0058	0.0069	0.0059	0.0045	0.0043	0.0040	0.0033	0.0047¹	0.0042	0.0038	0.0028	0.0034
	7		0.0249	0.0243	0.0243	0.0215	0.0230	0.0228¹	0.0256	0.0214	0.0221		
	8	0.0016	0.0020	0.0013	0.0013	0.0011	0.0007	0.0011					
	9		0.0076	0.0062	0.0053	0.0061	0.0059	0.0059	0.0044	0.0039			

(Injection of 20 mg. Pancreozymin between portions 4 and 5.)

¹ *Dog 6 & 7 received second doses of 14 & 20 mg. pancreozymin, respectively, after this sample.*
Dog 6, 11 kg. Secretin C: 5 mg/10 min. Dog 8, 10 kg. Secretin A: 3 mg/10 min.
Dog 7, 13 kg. Secretin C: 6.75 mg/10 min. Dog 9, 9 kg. Secretin A: 0.35 mg/10 min.
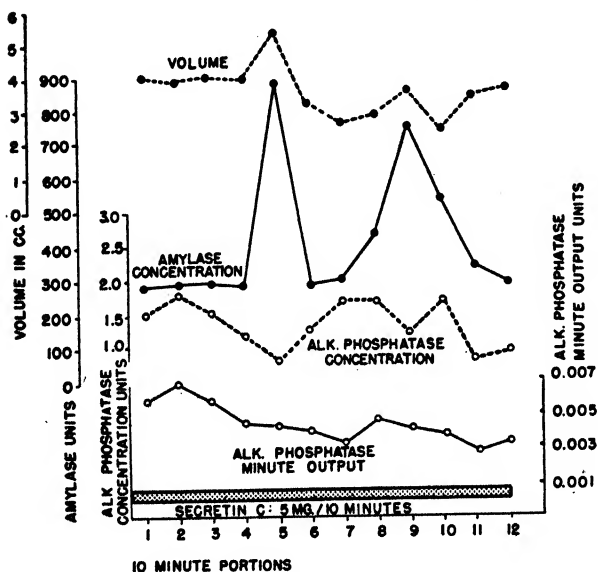
Fig. 5. *Dog 6*, 11 kg. Effect of pancreozymin on amylase and alkaline phosphatase. Twenty mg. of pancreozymin was injected after portion 4 and 14 mg. after portion 8.

DISCUSSION

It is well known that much of the earlier work on the action of secretin suffered from the use of relatively crude preparations containing either histamine-like sub-

stances or other vasodepressors which in themselves affect the pancreatic secretion. Of greater importance to this kind of study was the fact that many of these earlier secretin preparations contained varying amounts of pancreozymin.   Barrington's (9) finding that his secretin preparations stimulated enzyme secretion can be explained by the fact that he used SI, a secretion preparation which is known to contain pancreozymin.   Care was therefore taken to use in the present work highly purified secretin preparations in which the content of pancreozymin can be considered to be minimal or absent and which were shown to be free of vasodepressor action.

In the study of the effect of secretin on the enzyme production of the pancreas, the 'continuous' secretin injection provides an ideal method of approach because a continuous uniform secretion would serve to distinguish a 'washing out' process from a real production of enzymes.   The results observed clearly indicate that the increase of secretin administration did not bring about a continuous increase in enzyme output. Here, the effect of histamine on the pepsin response of gastric secretion (22) is in contrast.   The minute output of pepsin in response to large doses of histamine was at all times significantly larger (although the concentration of pepsin fell) than that in response to small doses and the effect of the higher dose of histamine, in increasing pepsin output, tended to continue after resumption of a lower dose of histamine.   It seems to be conclusive from our results that secretin has no stimulating effect on amylase production by the pancreas.

These results suggest that there is a constant basal rate of secretion of amylase (and probably of the other pancreatic enzymes).   When little or no water and bicarbonate are being secreted this amylase accumulates within the gland tubules, to be 'washed out' in the first portion of juice secreted after a period of quiescence. When secretin is the sole stimulus acting upon the pancreas, this basal rate of enzyme secretion continues unchanged and the concentration of amylase in the juice depends simply on the amount of water which the secretin causes the gland to secrete and thus to be available for dissolving the fixed amount of amylase.

We have previously shown that with the degeneration of the ductule cells that occurs in association with prolonged alloxan diabetes, a diminution in the volume of juice secreted in response to secretin occurs (24).   This finding suggested that these ductule cells might be the site where secretin acts to stimulate secretion of water and bicarbonate.   The present studies neither support nor disprove this possibility. They do show that at least one element of pancreatic juice, namely alkaline phosphatase, probably is secreted by the ductule cells.

It was shown in this work that both secretin and pancreozymin have no effect on alkaline phosphatase production in the pancreas.   This observation suggests that the source of enzyme production is probably not the same for phosphatase as for the other three major enzymes.   This supports Jacoby's proposal (16) that the ductule cells are responsible for the production of the phosphatase, whereas the acinar cells secrete the other three enzymes, namely the amylase, trypsinogen and lipase, since the alkaline phosphatase reaction is always positive in the ductule cells of the dog's pancreas, and negative in the glandular cells.

In the case of the secretion of alkaline phosphatase by the liver into the bile, there are two theories concerning the origin of the enzyme (23).   One theory holds that the alkaline phosphatase of the blood serum is excreted into the bile; the other states

that the alkaline phosphatase which appears in the bile is formed by the liver cells. No decision can be made as to which of these mechanisms applies to the secretion of alkaline phosphatase by the pancreas.

SUMMARY

The effect of secretin and pancreozymin on amylase and alkaline phosphatase secretion by the dog's pancreas has been observed by using highly purified secretin and pancreozymin. A 'continuous' secretin injection method was used. The results indicate that an increase of secretin administration causes an increase in the volume of juice but does not bring about an increase in the output of the enzyme per minute. Secretin, therefore, has no stimulating effect on enzyme production of the pancreas, neither amylase nor alkaline phosphatase. Pancreozymin stimulates the amylase output of the pancreas, but not alkaline phosphatase.

The observations indicate that the source of enzyme secretion is probably not the same for phosphatase as for the other three major enzymes. The proposal suggested by Jacoby (16) that the ductule cells are responsible for the production of the phosphatase and the acinar cells for the other three enzymes has thus been supported.

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DERIVATION OF LEADS I AND III IN THE DOG FROM AN ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VR, VL, AND VF[1]

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BEAM deflections in the standard limb leads I and III result when potential differences exist between the two extremity electrodes employed in recording each of these leads. By convention, upward movement of the beam in lead I occurs when the potential at the right forelimb is negative relative to that at the left forelimb, while movement of the beam downward occurs when the potential at the right forelimb is positive relative to that at the left. Similarly, in lead III upward movement of the beam occurs when the left forelimb is negative relative to the left hindlimb, while downward movement occurs when the potential at the left forelimb is positive relative to that at the left hindlimb. As Goldberger has pointed out, standard limb leads record only differences in potential between two given extremities and, therefore, on theoretical grounds, an upward movement of the beam will result when any one of five different combinations of potentials exists at a given moment at these extremities (1). Similarly, downward movement of the beam will occur when any one of five different combinations of potentials exists at the two extremities. When the beam remains at the isoelectric line in a standard limb lead during the depolarization process in a portion of the ventricle, the potentials developed at each of the two extremities must be of the same electrical sign and of equal magnitude. This will occur when any one of the following three possibilities exists: *a*) negative potentials of equal magnitude at both extremities; *b*) positive potentials of equal magnitude at both extremities; *c*) zero potential at both extremities.

Hoff, Nahum and Kaufman (2–5) have previously demonstrated that upward movements of the beam in lead I in the dog resulted mainly from depolarization of the posterior right ventricle, while downward movements resulted from depolarization of the anterior left ventricle. Depolarization of the remaining regions of the ventricles was found not to cause deflections of the beam in lead I. Similarly, upward movements of the beam in lead III were attributed to depolarization of the anterior right ventricle, while downward movements took place when the posterior left ventricle was depolarized. Depolarization occurring in the rest of the ventricles produced minimal or no effect upon beam movement in lead III. The entire ventricular complex in both leads I and III was considered by these investigators to result from the interaction of oppositely directed electrical forces derived from the depolarization of specific regions of the right and left ventricles, and that these regions included the entire thickness of the myocardium in either ventricle (6–8). This explanation of the genesis of the QRS complex in leads I and III, based upon experimental observations, differs from the conclusions reached by Gardberg and Ashman (9) who attempted to visualize the ventricular complex in terms of the advance and retreat of anatomically localized dipoles. They concluded that potentials resulting from excitation of the right ventricle are not recognizable as such in the electrocardiogram, except perhaps at the very onset or at the termination of ventricular excitation when the unopposed vectors resulting from the spread of the excitatory process initially through the septum from left to right and terminally in the pulmonary conus dominate and are thus responsible for the inscription of a Q-wave and S-wave, respectively. According to their analysis, it is the spread of excitation through the left ventricle from endocardium to epicardium which primarily accounts for both upstroke and downstroke in the ventricular complex in leads I and III. The experiments to be described below offer no support for the theoretical explanation given by Gardberg and Ashman for the genesis of the QRS complex in the standard limb leads, and confirm and extend the views first expressed by Hoff, Nahum and Kaufman.

Since any given beam movement recorded in the standard limb leads may result from any one of five different combinations of potentials existing at the two extremities employed in recording the lead, a more exact analysis of the derivation of leads I and III requires knowledge of the origin and nature of the instantaneous potentials developed independently at each limb throughout the cardiac cycle. This knowledge is now available from recent experimental studies in the dog on the nature of the 'unipolar' extremity leads VR, VL and VF (10–12). In those studies it was found that the ventricular complex in each 'unipolar' extremity lead represented the algebraic summation of oppositely directed potentials derived from the depolarization of specific proximal and distal ventricular zones. The spatial distribution of these ventricular zones was found to differ for each 'unipolar' extremity lead. Figure 1 shows a schematic representation of the heart surface as it was experimentally found to be divided into proximal, intermediate, and distal zones for each of the three 'unipolar' extremity leads. It can be seen that any segment of the heart which lies in the proximal zone of one extremity lead is also situated in the proximal, intermediate or distal zones of the other two extremity leads. It is possible to localize the site of preponderant depolarization during any given time interval to the proximal, intermediate or distal zone of a given 'unipolar' extremity lead from the direction of beam movement in that lead during that interval. Downward movement of the beam localizes the process to the proximal zone of the lead while upward movement of the beam localizes it to the distal zone. An isoelectric beam indicates either depolarization in the intermediate zone of the lead or equal degree of depolarization in both proximal and distal zones.

In any given 'unipolar' extremity lead, however, the proximal and distal zones are each relatively large and more specific localization within a given zone becomes possible only from study of the direction of beam movement in each of several 'unipolar' leads recorded simultaneously. For example, if during the same time interval the beam is moving downward in each of two leads, the depolarization process is more specifically localized to that region of the heart which lies in the proximal zone of both leads (all segments labelled *PP* in fig. 1). On the other hand, if the beam moves downward in one lead, but upward in the second lead, the site of preponderant depolarization is localized to that region of the heart which lies in the proximal zone of the first lead, but in the distal zone of the second (all segments labelled *PD* in fig. 1). By study of the three 'unipolar' extremity leads, VR, VL, and VF, recorded simultaneously, even more discrete localization becomes possible. For example, if during the same time interval the electrocardiographic beam is moving downward

in both VR and VL, but upward in VF, the depolarization process is localized to that segment of the heart which is labelled *PPD* in figure 1. Conversely, if the beam is moving upward in both VR and VL, but downward in VF, the process is localized to that segment of the heart labelled *DDP* in figure 1. By similar analysis, any combination of beam movements in VR, VL, and VF during any small time interval will localize the site of preponderant depolarization during that interval to one or another of the various segments depicted in figure 1.

It should be remembered that each 'unipolar' extremity lead records the instantaneous differences in potential which exist throughout the cardiac cycle between the exploring electrode and the central terminal electrode. Leads taken in such a manner may not be truly unipolar if the central terminal electrode is not at zero potential at all times during the cardiac cycle. However, if the potential of the central terminal electrode, whatever it may be, is considered at any moment during the cardiac cycle to be the same in each lead when these leads are recorded simultaneously, lead I may be derived from the equation lead I = lead VL − lead VR, and lead III may be derived from the equation lead III = lead VF − lead VL.

By definition:

1) Lead I　　= potential at left arm (LA)　　− potential at right arm (RA)
2) Lead III =　　"　　"　left leg (LL)　　−　　"　　"　left arm (LA)
3) Lead VR =　　"　　"　right arm (RA) −　　"　　"　central terminal
4) Lead VL =　　"　　"　left arm (LA)　　−　　"　　"　　"　　"
5) Lead VF =　　"　　"　left leg (LL)　　−　　"　　"　　"　　"

Equations 1 and *2* may therefore be rewritten as follows:

Lead I = (Lead VL + potential C.T.) − (Lead VR + potential C.T.)
Lead III = (Lead VF + potential C.T.) − (Lead VL + potential C.T.)

If the contribution of the central terminal to the potential differences recorded in each of the three 'unipolar' extremity leads is considered to be the same at any given moment when these leads are recorded simultaneously, it follows that Lead I = Lead VL − Lead VR and that Lead III = Lead VF − Lead VL.

Thus, knowing the nature of leads VR, VL, and VF, these equations should permit one to learn the reasons why normal and/or injury potentials from certain specific regions of the heart are maximally recorded in one or another of the standard limb leads I and III, while similar potentials from other specific regions are minimally or not at all recorded in one or another of these leads. Lead II is not analyzed in this study because by definition lead II = lead I + lead III.

The experimental methods employed in the following studies have been described in detail in previous communications concerning the nature of "unipolar" extremity leads (10, 11, 12).

DERIVATION OF LEAD I FROM ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VR
AND VL

A. Regions of the Heart in Which Depolarization Results in Maximal Deflections of the Beam in Lead I

Exploration of the ventricular surfaces of the heart by various techniques reveals that the greatest upward deflections of the beam in lead I occur when the mid-third of the posterior right ventricle is depolarized (segment 1, fig. 2A), while the greatest downward deflections in this lead result from depolarization of the mid-third of the anterior left ventricle (segment 6, fig. 2A). The explanation for these observed findings becomes apparent from a study of the changes that occur in leads VR and VL during the depolarization and repolarization of these heart segments.

1. Depolarization initiated in the mid-third of the posterior right ventricle by epicardial stimulation results in oppositely directed initial deflections in the extrasystoles recorded in VR and VL, indicating that the initial potentials developed at each of the forelimbs are of opposite electrical sign, the right forelimb being initially negative relative to the left. Lead I, therefore, exhibits an initial large upward deflection in the extrasystole (fig. 3A). Conversely, stimulation of the mid-third of the anterior left ventricle results in initial upward movement of the beam in VR, but in initial downward movement in VL, indicating that the right forelimb is
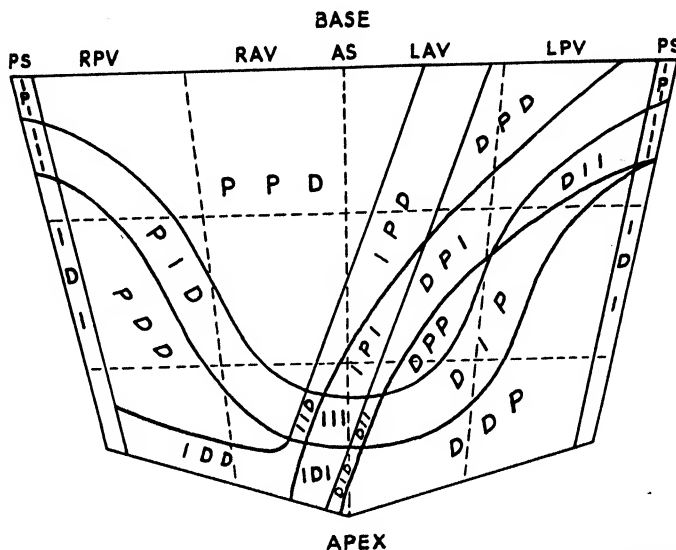
Fig. 1. SCHEMATIC DRAWING OF VENTRICULAR SURFACE OF DOG HEART showing overlapping of the proximal, intermediate and distal zones of leads VR, VL, and VF (composite picture of findings in 40 dogs). *PS*, posterior septum; *RPV*, right posterior ventricle; *RAV*, right anterior ventricle; *AS*, anterior septum; *LAV*, left anterior ventricle; *LPV*, left posterior ventricle; *P*, proximal zone; *I*, intermediate zone; *D*, distal zone. The various areas diagrammed are labelled according to their zonal representation in leads VR, VL, and VF. The first of the three letters which label each of the areas represents the zone of lead VR, the second letter the zone of lead VL, and the third letter the zone of lead VF (e.g., area labelled *PPD* indicates that this portion of the heart lies in the proximal zone of leads VR and VL, but in the distal zone of lead VF).

initially positive relative to the left forelimb. Lead I, therefore, exhibits an initial large downward deflection (fig. 3B).

2. M/5 KCl solution applied to the surface of the mid-third of the posterior right ventricle produces ST-segment elevation in VR and ST-segment depression in VL, indicating that injury to this segment of the heart results in the development during diastole of potentials at the right forelimb which are negative with respect to those developed at the left forelimb (13). Lead I reflects this relationship by upward displacement of the diastolic baseline, and therefore exhibits depression of the ST-segment (fig. 4A). When M/5 KCl solution is applied to the surface of the mid-third of the anterior left ventricle, VR exhibits ST-segment depression while VL exhibits ST-segment elevation, indicating that injury to this region results in the

development during diastole of potentials at the right forelimb which are positive relative to those developed at the left forelimb. In lead I, therefore, downward displacement of the diastolic baseline occurs and an ST-segment elevation is recorded (fig. 5A).
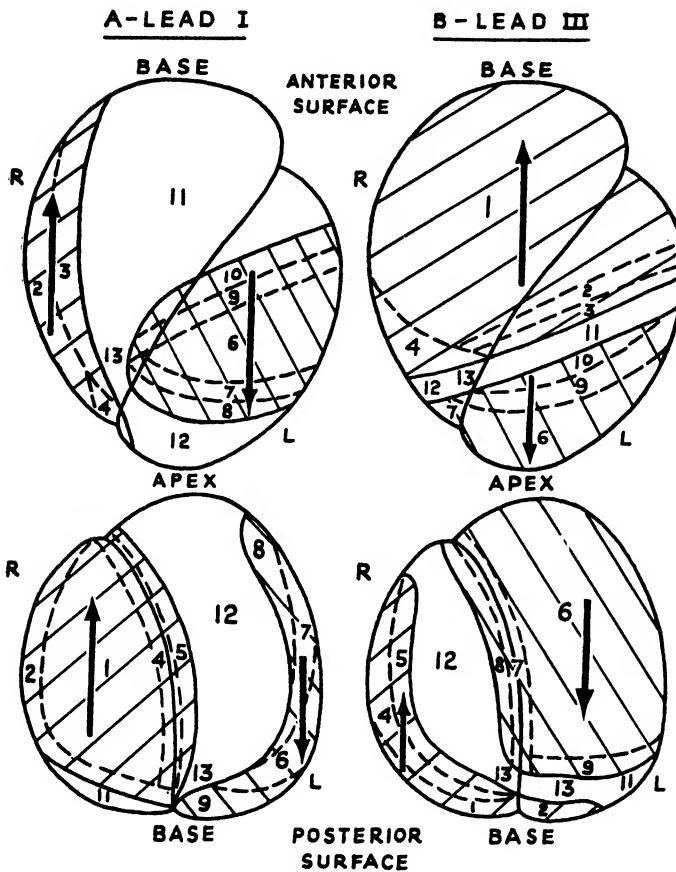
Fig. 2. DRAWING OF ANTERIOR AND POSTERIOR HEART SURFACE in the dog showing the electrically active and neutral regions for leads I and III. The lined areas represent the electrically active regions for leads I and III. The arrows point in the direction of beam movement in these leads when these regions are depolarized. The blank areas represent the electrically neutral regions for leads I and III. *A*. The numbers identify the specific segments whose depolarization produces the thirteen different combinations of potentials at the right and left forelimbs, as described in the text. *B*. The numbers identify the specific segments whose depolarization produces the 13 different combinations of potentials at the left forelimb and left hindlimb, as described in the text.

3. Acceleration of repolarization of the mid-third of the posterior right ventricle by heating results in increased positivity of the T-wave in VR, but in increased negativity of T-VL. The increase in the height of T-VR is due to increase in the magnitude of the positive potential developed at the right forelimb, while the deep inversion of the T-VL is due to increase in the magnitude of negative potential developed at the left forelimb. Inasmuch as the right forelimb is initially positive

with respect to the left, the beam in lead I is initially deflected downward, inscribing an inverted T-wave (fig. 6). Decelerating the repolarization of this region by cooling results in increased negativity of T-VR and in increased positivity of T-VL. Because the right forelimb becomes initially negative relative to the left, the beam in lead I
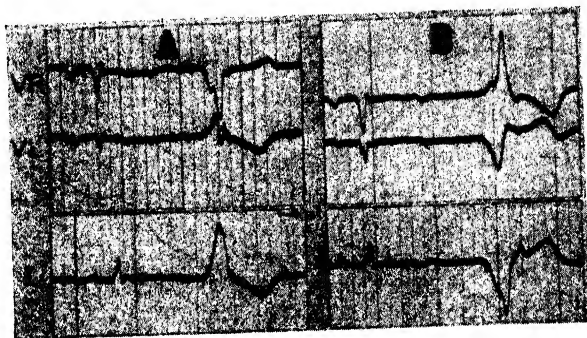
Fig. 3. 7.0 K. MALE DOG, April 8, 1948. *A. Forced extrasystoles from right posterior ventricle* The fine vertical lines represent 0.04-sec. time intervals. Following the stimulus artefact, the initial movement of the beam is downward in VR, upward in VL, and upward in lead I. *B. Forced extrasystoles from left anterior ventricle.* The initial movement of the beam is upward in VR, downward in VL and downward in lead I.
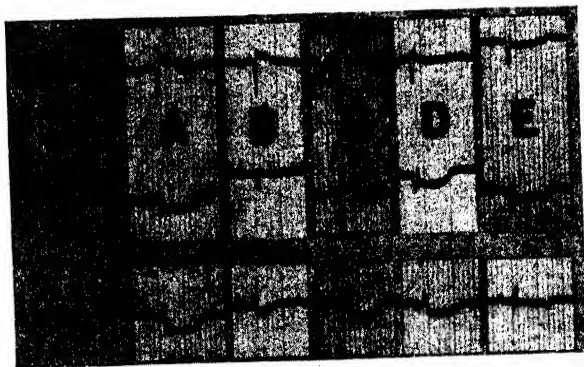
Fig. 4. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VR and VL that produce ST-segment depression in lead I. *A.* 5.6 K. female dog, Aug. 21, 1947. M/5 KCl applied to segment 1, fig. 2A. The ST-segment is elevated in VR, depressed in VL and sharply depressed in lead I. *B.* 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment 2, fig. 2A. The ST-segment is elevated in VR, isoelectric in VL and moderately depressed in lead I. *C.* 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 3, fig. 2A. The ST-segment is elevated in both VR and VL, but to a greater extent in VR. ST-segment in lead I moderately depressed. *D.* 5.6 K. female dog, Aug. 21, 1947. M/5 KCl applied to segment 4, fig. 2A. ST-segment isoelectric in VR, depressed in VL and depressed in lead I. *E.* 8.7 K. male dog, Aug. 7, 1947. M/5 KCl applied to segment 5, fig. 2A. ST-segment depressed in both VR and VL, but to a greater extent in VL. Minimal depresion of ST-I. Control records are not exhibited here, but all leads showed isoelectric ST-segments.

is initially deflected upward, inscribing an upright T-I (fig. 6). Alterations of the speed of repolarization by similar treatment of the mid-third of the anterior left ventricle results in opposite changes in T-VR, T-VL, and T-I (fig. 6).

The above experimental findings all lead to the same conclusion, namely, that depolarization of the mid-third of the posterior right ventricle results in the development of potentials at the right forelimb which are negative with respect to those
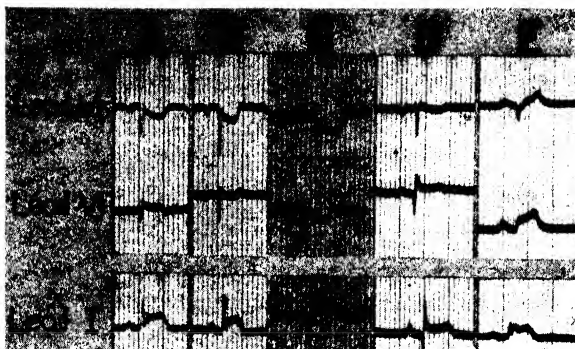
Fig. 5. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VR and VL that produce ST-segment elevation in lead I.   *A*. 7.2 K. female dog, Sept. 21, 1947.   M/5 KCl applied to segment 6, fig. 2A.   The ST-segment is depressed in VR, elevated in VL and elevated in lead I.   *B*. 6.7 K. male dog, Sept. 11, 1947.   M/5 KCl applied to segment 7, fig. 2A.   The ST-segment is depressed in VR, isoelectric in VL and elevated in lead I.   *C*. 9.6 K. male dog, July 31, 1947.   M/5 KCl applied to segment 8, fig. 2A.   The ST-segment is depressed in both VR and VL, but to a greater extent in VR.   ST-I is elevated.   *D*. 8.0 K. male dog, Sept. 26, 1947.   M/5 KCl applied to segment 9, fig.2A. The ST-segment is isoelectric in VR, moderately elevated in VL, and moderately elevated in lead I. *E*. 8.7 K. male dog, Aug. 7, 1947.   M/5 KCl applied to segment 10, fig. 2A.   The ST-segment is elevated in both VR and VL, but to a greater extent in VL.   ST-I is moderately elevated.   Control records are not exhibited, but all showed isoelectric ST-segments.

Fig. 6. CHANGES IN T-WAVE in leads VR, VL, and I following heating and cooling the heart surface. 7.2 kg. male dog, May 18, 1948. *RPV* = right posterior ventricle; *LAV* = left anterior ventricle; *RAV* = right anterior ventricle; *LPV* = Left posterior ventricle. Warming *RPV* makes T-VR more positive, T-VL more negative, and T-I sharply inverted. Cooling *RPV* makes T-VR more negative, T-VL more positive, and T-I sharply upright. Warming *LAV* makes T-VR more negative, T-VL more positive, and T-I sharply upright. Cooling *LAV* makes T-VR more positive, T-VL more negative, and T-I sharply inverted. Warming *RAV* makes both T-VR and T-VL more positive. T-I shows no change. Cooling *RAV* makes both T-VR and T-VL more negative. T-I shows no change. Cooling *RAV* makes both T-VR and T-VL more positive. T-I shows no change. Warming *LPV* makes both T-VR and T-VL more negative. T-I shows little change. Cooling *LPV* makes both T-VR and T-VL more positive. T-I shows only slight change.
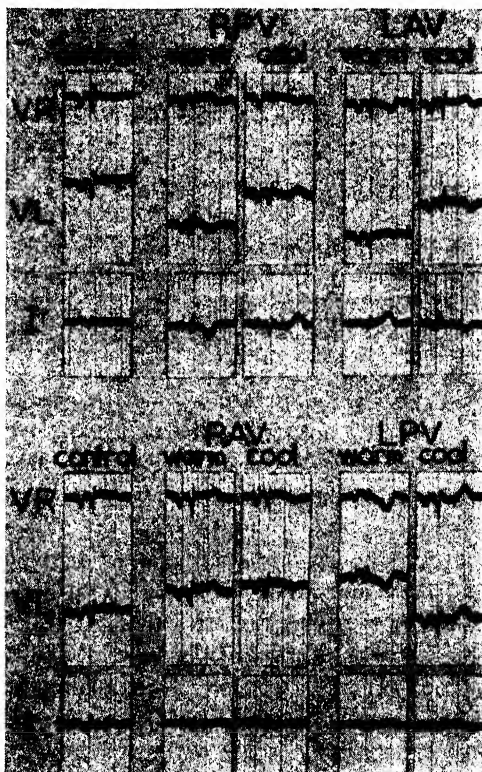
developed at the left forelimb, and that depolarization of the mid-third of the anterior left ventricle results in the development of potentials at the right forelimb which are

positive in relation to those developed at the left forelimb. Since in each case the potentials at the two extremities are of opposite electrical sign, lead I, recording the difference in potential between the two extremities, exhibits maximal deflections of the beam upward and downward respectively following the depolarization of these two heart segments.

B. *Regions of the Heart in Which Depolarization Results in Deflections of the Beam in Lead I of Lesser Magnitude Than Those Described in Section A*

Upward deflections of the beam in lead I have also been found to result when those regions of the heart which border immediately upon the mid-third of the posterior right ventricle become depolarized, while downward deflections also result from depolarization of those regions that border upon the mid-third of the anterior left ventricle, but these deflections are of lesser magnitude than those which result from depolarization of the mid-third of the posterior right and anterior left ventricles. Study of the nature of the ST-segment displacements in leads VR, VL, and I resulting from experimental injury to these specific regions furnishes the explanation for these observations.

1. M/5 KCl solution applied to the epicardial surface of heart segment 2, figure 2A, produces ST-segment elevation in VR but no displacement of ST-VL, indicating that during diastole the right forelimb is negative relative to the left. Lead I reflects this relationship by an upward displacement of the diastolic baseline and hence exhibits a depressed ST-segment (fig. 4B).

2. M/5 KCl solution applied to heart segment 3, figure 2A, produces ST-segment elevation in both VR and VL, but the degree of upward displacement of the ST-segment is greater in VR than in VL, indicating that the degree of negativity at the right forelimb during diastole is greater than that at the left forelimb. During diastole the right forelimb is negative relative to the left, and therefore lead I exhibits upward displacement of the diastolic baseline (ST-segment depression, fig. 4C).

3. M/5 KCl solution applied to segment 4, figure 2A, produces no displacement of ST-VR, but depression of ST-VL, indicating that during diastole the right forelimb is negative relative to the left. Lead I, therefore, exhibits ST-segment depression (fig. 4D).

4. M/5 KCl solution applied to segment 5, figure 2A, produces ST-segment depression in both VR and VL, but the degree of depression is somewhat greater in VL than in VR. Although both extremities are at positive potential during diastole, the right forelimb is somewhat negative relative to the left, and lead I, therefore, exhibits very slight depression of the ST-segment (fig. 4E).

5. M/5 KCl solution applied to segment 7, figure 2A, produces depression of ST-VR but no displacement of ST-VL, indicating that during diastole the right forelimb is positive relative to the left. In lead I, therefore, there is downward displacement of the diastolic baseline (ST-segment elevation, fig. 5B).

6. M/5 KCl solution applied to segment 8, figure 2A, produces depression of the ST-segment in both VR and VL, but the degree of downward displacement is greater in VR than in VL. The right forelimb is thus relatively positive with respect to the left during diastole, and lead I therefore exhibits downward displacement of the diastolic baseline (ST-segment elevation, fig. 5C).

7. M/5 KCl solution applied to segment 9, figure 2A, produces no displacement of ST-VR, but produces moderate elevation of ST-VL. The right forelimb is thus positive relative to the left during diastole, and therefore lead I exhibits slight ST-segment elevation (fig. 5D).

8. M/5 KCl solution applied to segment 10, figure 2A, produces ST-segment elevation in both VR and VL, but the degree of upward displacement is greater in VL than in VR. The right forelimb is thus somewhat positive relative to the left during diastole, and lead I therefore exhibits moderate ST-segment elevation (fig. 5E).

The five possible combinations of positive, zero, and negative potentials at the right and left forelimbs that could account theoretically for an upward movement of the beam in lead I have all been found to exist and each combination has been demonstrated to result from the depolarization of specific portions of the posterior right ventricle, posterior right apex, right lateral wall, and the posterior septum (segments 1–5, fig. 2A). The greatest difference in potential between the two extremities as recorded in lead I has been shown to develop following depolarization of the mid-third of the posterior right ventricle (segment 1, fig. 2A) while differences of lesser magnitude, but of the same electrical sign, result from the depolarization of the immediately adjacent regions. Similarly, the five possible combinations of potential at the right and left forelimbs that could account theoretically for a downward movement of the beam in lead I have each been demonstrated to result from the depolarization of specific portions of the anterior left ventricle, the mid-third of the anterior septum, a small portion of the lower third of the anterior right ventricle near the septum, the upper two-thirds of the lateral wall of the left ventricle, and a small segment of the basal posterior left ventricle (segments 6–10, fig. 2A). The mid-third of the anterior left ventricle (segment 6, fig. 2A) was found to be the region whose depolarization results in the development of the greatest difference in potential between the right and left forelimbs, while depolarization of the immediately surrounding regions results in differences of lesser magnitude, but of similar electrical sign.

More accurate localization of injuries to the dog heart is possible from study of the ST-segment displacements in leads VR and VL than from a study of ST-segment displacement in lead I alone. ST-segment elevation in lead I localizes the injury only to the general region of the anterior left ventricle, while the direction of ST-segment displacements in VR and VL helps localize the injury to more specific parts of this general region. ST-segment depression in lead I localizes the injury only to the general region of the posterior right ventricle, while displacements of ST-VR and ST-VL help localize the injury to more specific parts of this region.

C. Regions of the Heart in Which Depolarization Results in Minimal or no Deflections of the Beam in Lead I

Exploration of the ventricular surfaces of the heart reveals that there exist specific regions in which depolarization fails to produce any appreciable deflection of the beam in lead I. These segments are the mid and upper thirds of the anterior right ventricle, the upper third of the anterior septum, the basal portion of the anterior left ventricle, the left apex, and the lower two thirds of the posterior left ventricle (segments 11–13, fig. 2A). As has been mentioned above, there are three

possible conditions in which an isolectric beam can occur in a standard limb lead even though certain segments of the heart are the site of depolarization at the moment. In each instance there is no appreciable potential difference developed between the two extremities as a result of such depolarization. The reasons for the relative or absolute lack of electrical representation in lead I of the heart segments listed above may be determined from a study of the changes occurring in leads VR and VL following excitation, injury, or alteration in rate of repolarization of these segments.

1. Mid and upper thirds of anterior right ventricle (segment 11, fig. 2A). a) M/5 KCl solution applied to this region produces ST-segment elevation of the same degree in both VR and VL, indicating that the potentials which develop at each extremity during diastole as a result of the current of injury are each negative and of the same magnitude. Because there is no difference in potential between the two limbs during diastole, the ST-segment in lead I remains isoelectric (fig. 7A).

b) Acceleration of the rate of repolarization of this segment by heat makes the T-wave become more upright in both VR and VL. Because the degree of
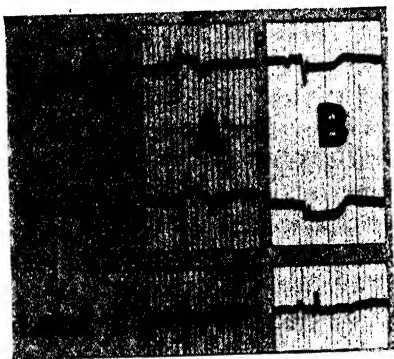
Fig. 7. ST-SEGMENT DISPLACEMENTS in VR and VL that fail to produce ST-segment change in lead I. *A.* 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 11, fig. 2A. The ST-segment is elevated to the same extent in both VR and VL. The ST-segment in lead I remains isoelectric. *B.* 5.6 K. male dog, Aug. 21, 1947. M/5 KCl applied to segment 12, fig. 2A. The ST-segment is depressed to the same extent in both VR and VL. The ST-segment in lead I remains isoelectric. Control records are not exhibited, but all leads showed isoelectric ST-segments.

change in the amplitude of the T-wave in each of these leads tends to be of equal magnitude, the difference in potential between the two forelimbs throughout the repolarization process after treatment with heat remains the same as that which existed before the heating. The amplitude and direction of the T-wave in lead I therefore tends to remain unchanged despite the fact that the rate of repolarization of a large portion of the heart has been materially altered (fig. 6). Conversely, cooling this region results in inversion of the T-waves in both VR and VL, but because the degree of change is the same in each of these leads, the T-wave in lead I tends to remain unaltered (fig. 6).

2. Left apex and lower two-thirds of posterior left ventricle (segment 12, fig. 2A). a) M/5 KCl solution applied to this region produces ST-segment depression of the same degree in both VR and VL, indicating that positive potentials of equal magnitude are developed at each forelimb during diastole as a result of the injury current. No difference in potential has developed between the two extremities during diastole, and therefore lead I reveals an isoelectric ST-segment (fig. 7B).

b) When extrasystoles were elicited by stimulation of the epicardial surface of the lower third of the posterior left ventricle, the beam in lead I remained at the iso-

electric line for about 0.04 sec. before moving downward (fig. 8). During this 0.04-sec. interval, however, the beam in lead III rapidly moved downward. The explanation for this isopotential period in lead I (at a time when some parts of the heart were undergoing activation) becomes clear from an analysis of the configuration of the extrasystole as it was simultaneously inscribed in VR and VL. It can be seen in both VR and VL that the beam initially moved upwards with identical slopes, indicating that the potentials developed at each forelimb at each instant during this 0.04-sec. period were of the same electrical sign and of equal magnitude. Because no difference in potential developed between the two extremities as a result of the early activation of the posterior left ventricle, the beam in lead I, therefore, remained at the isoelectric line until the excitatory process had spread to other parts of the heart.

c) When repolarization of this heart segment is accelerated by heat, the T-wave in both VR and VL becomes more negative, the degree of change in the T-wave in

Fig. 8. FORCED EXTRASYSTOLE from lower third of posterior left ventricle. 9.0 K. male dog, Oct. 14, 1947. The initial movement of the beam is upward in both VR and VL and the slope of the upward deflection is the same in each lead during the 0.04-sec. period following the stimulus artefact. The initial movement of the beam is downward in lead VF and immediately follows the stimulus artefact. The beam remains at the isoelectric line in lead I until 0.04 sec. after the stimulus artefact. In lead III the beam is deflected downward immediately after the stimulus artefact.
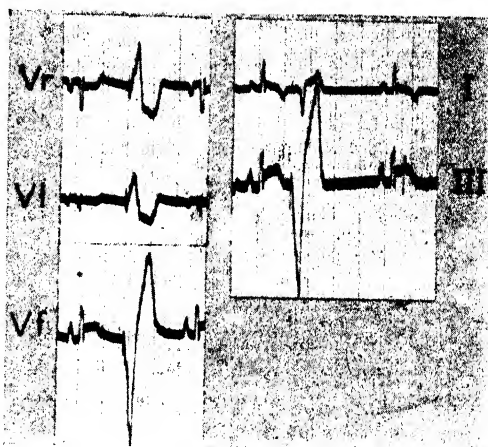
each of these leads being equal (fig. 6). The T-wave in lead I exhibits little or no alteration from the control value since the difference in potential between the two extremities throughout the repolarization process remains at the pretreatment level. Conversely, when repolarization of this region is delayed by cooling, both T-VR and T-VL become more upright. Because the degree of change in potential at both extremities tends to be of similar magnitude, the T-wave in lead I exhibits little or no change (fig. 6).

3. *Basal posterior left ventricle near the septum (segment 13, fig. 2A).* M/5 KCl solution applied to this segment produces no significant displacement of the ST-segment in either VR or VL, and therefore lead I exhibits an isoelectric ST-segment (fig. 9A, B). The reason for the lack of any displacement of the ST-segment in either VR or VL is that the injured region lies in the intermediate zone of each of these leads and is, therefore, in an electrically neutral area of both of these leads (fig. 1). In lead III, however, the ST-segment is displaced upward because the treated area lies within the proximal zone of lead VF (fig. 9A, B).

The above observations all demonstrate that potentials resulting from the

depolarization or repolarization of the major portion of the anterior right ventricle, the left apex, and the major portion of the posterior left ventricle fail to produce significant beam deflections in lead I.
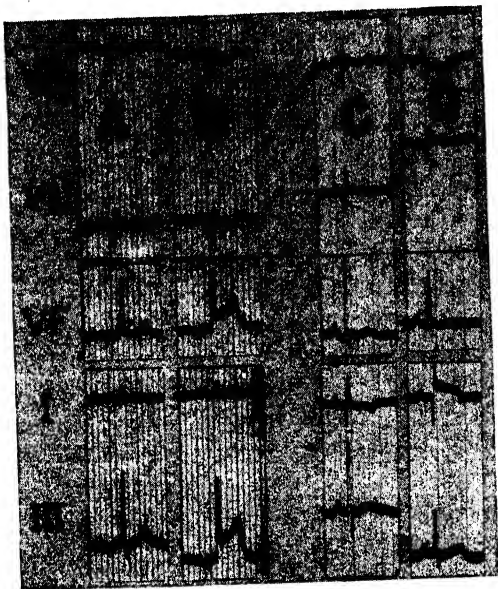
Fig. 9. REGIONS OF THE HEART in which injury produces ST-segment displacement in only one of the three unipolar leads. *A.* Control; 11.4 male dog, Oct. 14, 1947. *B.* M/5 KCl applied to segment 13, fig. 2A. The ST-segment tends to remain at the iso-electric line in VR and VL and lead I. The ST-segment is elevated in VF and lead III. *C.* Control; 6.5 K. male dog, Oct. 23, 1947. *D.* M/5 KCl applied to segments 1–3, 6, 9–11, fig. 2B. The ST-segment is depressed in VR, and isoelectric in VL and VF. Lead I exhibits ST-segment elevation. Lead III exhibits an isoelectric ST-segment.

SUMMARY AND CONCLUSIONS

The present study of the nature of lead I by analysis of the 'unipolar' extremity leads VR and VL permits the following conclusions:

1. The five possible combinations of potentials at the right and left forelimbs that could account theoretically for upward movement of the beam in lead I and the five possible combinations of potentials at these extremities that could account theoretically for downward movement of the beam in lead I have been shown to exist experimentally and each combination can be related to the depolarization of a specific ventricular region.

2. It is only when the site of preponderant depolarization lies within the anterior left ventricle that downward deflection of the beam occurs in the ventricular complex in lead I because only depolarization in this region results in the development of potentials at the right forelimb which are positive relative to those developed at the left forelimb. If a portion of this region is activated in advance of the rest of the ventricles, the initial movement of the beam in the ventricular complex will be downward and a Q-wave will, therefore, be inscribed in lead I. Later excitation of this region will also result in downward movement of the beam in lead I and this accounts for the downstroke of the R- and S-waves in this lead.

3. It is only when the site of preponderant depolarization lies within the posterior right ventricle and posterior septum that upward deflections of the beam occur in the ventricular complex in lead I because only depolarization of these regions gives rise to potentials at the right forelimb which are negative relative to those developed at the left forelimb. If a portion of these regions is activated early, the beam will move

upward and inscribe the upstroke of the Q- and R-waves in lead I.   If a part of these regions is the last to be activated, the beam will again be deflected upward in lead I, thus inscribing the upstroke of the S-wave in this lead.

4. No deflections of the beam take place in lead I when the major portions of the anterior right ventricle, the left apex, and the posterior left ventricle become depolarized or repolarized, since during these events no appreciable difference in potential develops between the right and left forelimbs.   Depolarization of the major portion of the anterior right ventricle results in the development of negative potentials of equal or approximately equal magnitude at both extremities, whereas depolarization of the major portion of the posterior left ventricle and left apex results in the development of positive potentials of equal or approximately equal magnitude at both extremities.   There exists a small segment of the basal posterior left ventricle near the septum in which depolarization fails to produce beam movements in lead I because it fails to produce any deflections of the beam in either VR or VL.

5. An isopotential ST-segment in lead I can occur when any of the following conditions exists: *a*) no current of injury is present; *b*) injury is present in the mid-third of the anterior right ventricle and/or the lower two-thirds of the posterior left ventricle and left apex; *c*) repolarization has not yet begun in the anterior left and posterior right ventricles; *d*) the anterior left and posterior right ventricles are repolarizing at the same rate, thus producing potentials at each extremity of the same magnitude but of opposite electrical sign so that the net potential at each extremity is zero.

6. More specific localization of injury to the heart is possible from study of ST-segment displacements in VR and VL than from study of ST-segment displacement in lead I alone.   ST-segment elevation in lead I localizes the injury only to the general region of the anterior left ventricle, while the displacements of the ST-segments in VR and VL help localize the injury to more specific parts of this region. ST-segment depression in lead I localizes injury only to the general region of the posterior right ventricle, while the displacements of the ST-segments in VR and VL help localize the injury to more specific parts of this region.

7. When the anterior left ventricle repolarizes in advance of the posterior right ventricle, the T-wave is upright in lead I because of the development of initial relative negativity at the right forelimb.   Conversely, when the posterior right ventricle repolarizes in advance of the anterior left ventricle, the T-wave in lead I is inverted because of the development of initial relative positivity at the right forelimb. The repolarization of the major portions of the anterior right and posterior left ventricles contributes little or nothing to the configuration of the T-wave in lead I because the repolarization of each of these regions tends to produce simultaneous potentials of the same magnitude and electrical sign at the right and left forelimbs.

These conclusions as to the derivation of lead I in the dog correspond closely with the findings of Hoff, Nahum and Kaufman (2–5) on the nature of the various components of the ventricular complex in this lead.   These authors described lead I as the algebraic summation of potentials derived mainly from the depolarization of the posterior right and anterior left ventricles.   They found that the depolarization of the anterior right and posterior left ventricles was not represented in lead I.   The

present study has more exactly delimited the electrically active as well as the electrically neutral areas for lead I, and has established the basis for the presence or absence of electrical representation of the various regions of the heart in lead I.

DERIVATION OF LEAD III FROM ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VF AND VL

A. Regions of the Heart in Which Depolarization Results in Maximal Deflections of the Beam in Lead III

Exploration of the ventricular surfaces of the dog heart reveals that the greatest upward deflections of the beam in lead III occur when the upper half of the anterior right ventricle (segment 1, fig. 2B) becomes depolarized while the greatest downward deflections in this lead result when the left apex and lower two thirds of the posterior
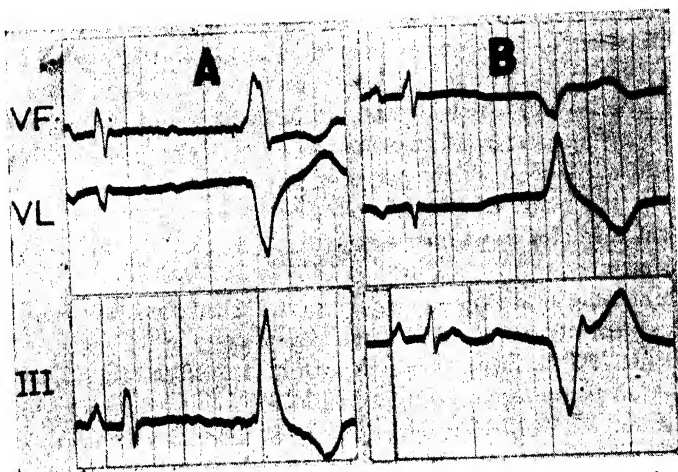
Fig. 10. 7.0 K. MALE DOG, Apr. 8, 1948. *A. Forced extrasystoles from anterior right ventricle.* The fine vertical lines represent 0.04-sec. time intervals. Following the stimulus artefact, the initial movement of the beam is upward in lead VF, downward in lead VL and upward in lead III. *B. Forced extrasystoles from posterior left ventricle.* Following the stimulus artefact the initial movement of the beam is downward in lead VF, upward in lead VL and downward in lead III.

left ventricle are depolarized (segment 6, fig. 2B). The explanation for these observations becomes apparent from a study of the changes that occur in the 'unipolar' extremity leads VF and VL following the depolarization and repolarization of these segments.

    1. Depolarization initiated in the anterior right ventricle by epicardial stimulation results in oppositely directed initial deflections of the beam in VF and VL, indicating that the potentials developed at each extremity are initially of opposite electrical sign, the left forelimb being initially negative with respect to the left hindlimb. Lead III, therefore, exhibits an initial upward deflection (fig. 10A). Conversely, stimulation of the left apex and lower two thirds of the posterior left ventricle results in initial downward deflection of the beam in VF but in initial upward movement of the beam in VL, indicating that the left forelimb is initially positive with respect to the left hindlimb. Lead III, therefore, exhibits an initial downward deflection (fig. 10B).

2. M/5 KCl solution applied to the surface of the anterior right ventricle (segment 1, fig. 2B) produces ST-segment elevation in VL and ST-segment depression in VF, indicating that injury to this region results in the development during diastole of potentials at the left forelimb which are negative relative to those developed at the left hindlimb. Lead III, therefore, exhibits an upward displacement of the diastolic baseline or ST-segment depression (fig. 11A). When M/5 KCl solution is applied to the surface of the left apex or lower two-thirds of the posterior left ventricle (segment 6, fig. 2B), ST-segment depression occurs in VL, while ST-segment elevation occurs in VF. This indicates that injury to this region of the heart results in the development during diastole of potentials at the left forelimb which are
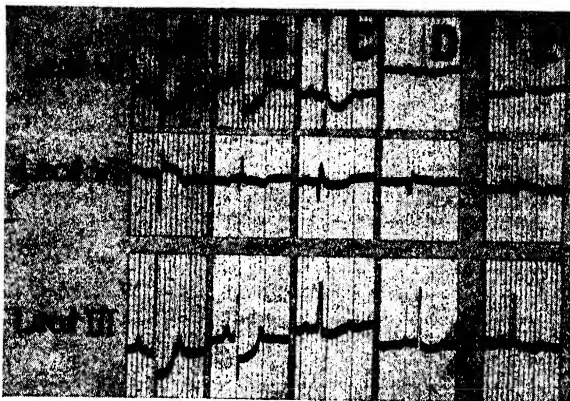
Fig. 11. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VF and VL that produce ST-segment depression in lead III. *A.* 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 1, fig. 2B. The ST-segment is depressed in VF, elevated in VL and depressed in lead III. *B.* 7.5 male dog, Jan. 6, 1948. M/5 KCl applied to segment 4, fig. 2B. The ST-segment is depressed in VF, isoelectric in VL and depressed in lead III. *C.* 8.0 K. male dog, Sept. 26, 1947. M/5 KCl applied to segment 5, fig. 2B. The ST-segment is depressed both in VF and VL, but to a greater extent in VF. ST-III is moderately depressed. *D.* 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment, 2, fig. 2B. The ST-segment is isoelectric in VF, moderately elevated in VL and moderately depressed in lead III. *E.* 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment 3, fig. 2B. The ST-segment is elevated both in VF and VL, but to a slightly greater extent in VL. ST-III shows minimal depression. Control records are not exhibited but all leads showed isoelectric ST-segments.

positive with respect to those developed at the left hindlimb. In lead III, therefore, the diastolic baseline is displaced downward and ST-segment elevation results (fig. 12A).

3. Acceleration of repolarization of the upper half of the anterior right ventricle by heat results in increased positivity of the T-wave in VL, but in increased negativity of T-VF. The increase in the height of the T-wave in VL is due to increase in the magnitude of the positive potential developed at the left shoulder, while the deep inversion of T-VF is due to increase in the magnitude of negative potential developed at the left hindlimb. Because the left forelimb is initially positive with respect to the left hindlimb, the beam in lead III is initially deflected downward, inscribing an inverted T-wave (fig. 13). Conversely, decelerating the repolarization of this region by cooling results in increased negativity of T-VL, but in increased positivity of T-
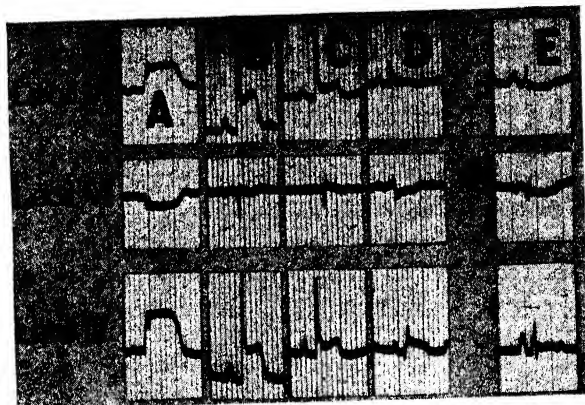
Fig. 12. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VF and VL that produce ST-segment elevation in lead III. *A.* 5.6 K. male dog, Aug. 21, 1947. M/5 KCl applied to segment 6, fig. 2B. The ST-segment is sharply elevated in VF, sharply depressed in VL and sharply elevated in lead III. *B.* 6.7 K. male dog, Sept. 11, 1947. M/5 KCl applied to segment 9, fig. 2B. The ST-segment is sharply elevated in VF, isoelectric in VL, and sharply elevated in lead III. *C.* 6.7 K. male dog, Sept. 11, 1947. M/5 KCl applied to segment 10, fig. 2B. The ST-segment is moderately elevated in both VF and VL, but to a greater extent in VF. ST-III is moderately elevated. *D.* 8.7 K. male dog, Aug. 7, 1947. M/5 KCl applied to segment 7, fig. 2B. The ST-segment is isoelectric in VF, moderately depressed in VL, and moderately elevated in lead III. *E.* 7.2 K. male dog, May 18, 1947. M/5 KCl applied to segment 8, fig. 2B. The ST-segment is slightly depressed in both VF and VL, but to a greater extent in VL. ST-III shows slight elevation. Control records are not exhibited, but all leads showed isoelectric ST-segments.
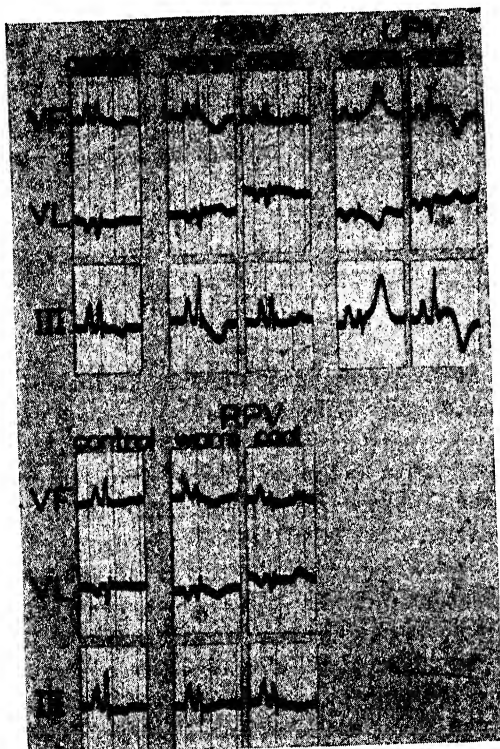
Fig. 13. CHANGES IN THE T-WAVE in leads VF, VL and III following heating and cooling the heart surface. 7.2 K. male dog, May 18, 1948. *RAV* = right anterior ventricle; *LPV* = left posterior ventricle; *RPV* = right posterior ventricle. Warming *RAV* makes T-VF more negative, T-VL more positive, and T-III sharply inverted. Cooling *RAV* makes T-VF more positive, T-VL negative, and T-III sharply upright. Warming *LPV* makes T-VF more positive, T-VL more negative, and T-III sharply upright. Cooling *LPV* makes T-VF more negative, T-VL more positive, and T-III deeply inverted. Warming *RPV* makes both T-VF and T-VL more negative. T-III shows only moderate change. Cooling *RPV* makes both T-VF and T-VL more positive. T-III shows only slight change. Changes in the QRS complex are noted, and will be discussed elsewhere.

VF.   Since the left forelimb is initially negative with respect to the left hindlimb during the inscription of the T-wave, the beam in lead III is initially deflected upward, inscribing an upright T-III (fig. 13).   Similar treatment of the left apex and posterior left ventricle with heat and cold results in changes in T-VL, T-VF, and T-III of opposite nature to those described above following treatment of the anterior right ventricle (fig. 13).

The above experimental findings all lead to the same conclusion, namely, that depolarization of the anterior right ventricle results in the development of negativity at the left forelimb relative to the left hindlimb, while depolarization of the left apex and lower two-thirds of the posterior left ventricle results in the development of positivity at the left forelimb relative to the left hindlimb.   Since in each case the potentials at the two extremities are of opposite electrical sign, lead III—recording the difference in potential between the two extremities—exhibits maximal upward or downward deflections of the beam respectively following the depolarization of these regions of the heart.

B. *Regions of the Heart in Which Depolarization Results in Deflections of the Beam in Lead III of Lesser Magnitude Than Those Described in Section A*

Upward movements of the beam in lead III also occur when regions of the heart which border immediately upon the upper half of the anterior right ventricle become activated, while downward deflections of the beam in lead III also occur when regions of the heart which border immediately upon the left apex ..nd lower two thirds of the posterior left ventricle become activated, but these deflections are of lesser magnitude than those described above in Section A.   Study of ST-segment displacements in leads VL, VF and III resulting from experimental injury to these regions furnishes the explanation for these observations.

1.   M/5 KCl solution applied to segment 2, figure 2B, produces no displacement of the ST-segment in VF, but upward displacement of the ST-segment in VL, indicating that during diastole the left forelimb is negative relative to the left hindlimb. The diastolic baseline in lead III is therefore displaced upward and an ST-segment depression is inscribed (fig. 11D).

2.   M/5 KCl solution applied to segment 3, figure 2B, produces slight upward displacement of the ST-segment in both VF and VL, but the degree of upward displacement is somewhat greater in VL than in VF, indicating that during diastole the left forelimb is negative relative to the left hindlimb.   The diastolic baseline in lead III is, therefore, displaced upward, and a minimal ST-segment depression results (fig. 11E).

3.   M/5 KCl solution applied to segment 4, figure 2B, produces downward displacement of the ST-segment in VF .but no displacement of ST-VL, indicating that the left forelimb is negative relative to the left hindlimb during diastole.   Lead III, therefore, exhibits a depressed ST-segment (fig. 11B).

4.   M/5 KCl solution applied to segment 5, figure 2B, produces depression of the ST-segment in both VF and VL, but the degree of depression is greater in VF than in VL.   The left forelimb is relatively negative with respect to the left hindlimb during diastole and, therefore, lead III exhibits slight depression of the ST-segment (fig. 11C).

5. M/5 KCl solution applied to segment 7, figure 2B, produces no displacement of ST-VF and depression of ST-VL. The left forelimb is positive relative to the left hindlimb during diastole, and, therefore, lead III shows elevation of the ST-segment (fig. 12D).

6. M/5 KCl solution applied to segment 8, figure 2B, produces slight depression of the ST-segments in both VF and VL, but the degree of depression is somewhat greater in VL than in VF. The left forelimb is positive relative to the left hindlimb during diastole and lead III therefore exhibits slight ST-segment elevation (fig. 12E).

7. M/5 KCl solution applied to segment 9, figure 2B, produces elevation of the ST-segment in VF, but no change of ST-VL. The left forelimb is positive relative to the left hindlimb during diastole and lead III exhibits ST-segment elevation (fig. 12B).

8. M/5 KCl solution applied to segment 10, figure 2B, produces elevation of the ST-segment in both VF and VL, but the degree of elevation is somewhat greater in VF than in VL. Lead III, therefore, exhibits moderate elevation of the ST-segment (fig. 12C).

Thus it has been demonstrated above that the depolarization of the upper two thirds of the anterior right ventricle, the upper two thirds of the anterior septum, the upper third of the anterior left ventricle, and a small portion of the basal posterior left ventricle (segments 1–4, fig. 2B) results in the development of negativity at the left forelimb relative to the left hindlimb and thus produces upward movement of the beam in lead III. Depolarization of the left apex, the lower third of the anterior left ventricle, a portion of the right apex, and the lower two thirds of the posterior left ventricle and septum (segments 6–10, fig. 2B) results in the development of negativity at the left hindlimb relative to the left forelimb and thus produces downward movement of the beam in lead III.

More specific localization of the site of injury to the heart can be obtained from a study of ST-segment displacements in leads VF and VL than from a study of ST-segment displacements in lead III alone. Upward displacement of the ST-segment in lead III localizes the injury only to the general region of the left apex and posterior left ventricle, while the displacements in leads VF and VL help localize the injury to more specific parts of this general region. Depression of the ST-segment in lead III localizes injury only to the general region of the anterior right ventricle, while the displacements in leads VF and VL help localize the injury to more specific parts of this region.

C. *Regions of the Heart in Which Depolarization Results in Minimal or no Deflection of the Beam in Lead III*

Exploration of the ventricular surfaces of the heart reveals that depolarization of major portions of the posterior right ventricle and the anterior left ventricle fails to produce significant deflection of the beam in lead III. It has been demonstrated above that depolarization of these segments of the heart is maximally recorded in lead I. The reasons for the electrical neutrality of these regions in lead III are revealed from study of changes occurring in leads VF and VL following injury to these segments or following alterations in their rates of repolarization.

1. Mid-third of posterior right ventricle (segment 12, fig. 2B). *a*) M/5 KCl solution applied to this region produces ST-segment depression of equal degree in both VF and VL, indicating that both the left forelimb and left hindlimb are at the same positive potential during diastole as a result of the current of injury. Since there is no difference in potential between the two extremities during diastole, lead III therefore exhibits an isoelectric ST-segment (fig. 14A).
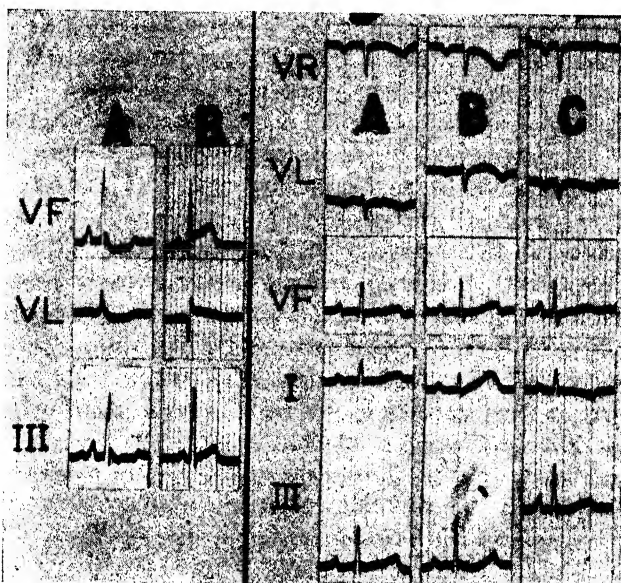
Fig. 14 *(left)*. ST-SEGMENT DISPLACEMENTS in VF and VL that fail to produce ST-segment changes in lead III. *A*. 7.0 K. female dog, June 2, 1948. M/5 KCl applied to segment 12, fig. 2B. The ST-segment is depressed to the same extent in both VF and VL. The ST-segment in lead III remains isoelectric. *B*. 11.4 K. male dog, Aug. 15, 1947. M/5 KCl applied to segment 11, fig. 2B. The ST-segment is elevated to the same extent in both VF and VL. The ST-segment in lead III remains isoelectric. Controls showed isoelectric ST-segments in all leads, but are not exhibited.

Fig. 15 *(right)*. CHANGES IN THE T-WAVE in leads VR, VL, VF, I, and III following heating and cooling the anterior left ventricle. 6.1 K. male dog, June 3, 1948. *A* = control; *B* = warming; *C* = cooling. Warming produces increased negativity of T-VR, but little change in T-VL and T-VF. T-I becomes more upright while T-III shows minimal change. Cooling produces upright T-VR, but little change in T-VF and T-VL. T-I becomes inverted while T-III remains essentially unaltered.

b) Acceleration of repolarization of this segment by heat makes the T-wave become more negative in both VF and VL. Because the degree of change in each of these leads tends to be of equal magnitude, the T-wave in lead III exhibits only moderate change in its configuration following the application of heat to this region (fig. 13). When the repolarization of this region is delayed by cooling it, both T-VF and T-VL become more positive. Because the degree of change in the T-wave in each of these leads tends to be similar, lead III fails to exhibit any significant change in T-wave configuration (fig. 13).

2. Mid-third of anterior left ventricle (segment 11, fig. 2B). M/5 KCl solution applied to this segment produces slight ST-segment displacement upward of equal degree in both VF and VL. Since the potentials which exist during diastole at both

the left forelimb and left hindlimb are of the same electrical sign and magnitude, lead III exhibits no displacement of the ST-segment (fig. 14B). These changes occur in VF and VL when the injury involves chiefly that portion of the mid-third of the anterior left ventricle which lies in the proximal zones of both of these leads (segment labelled *DPP*, fig. 1).

3. Major portion of anterior left ventricle (segments 1-3, 6, 9-11, fig. 2B). a) M/5 KCl solution applied over the entire surface of the anterior left ventricle may also produce no appreciable displacement of the ST-segment in either VF or VL, and as a consequence there will be no change in the ST-segment in lead III (fig. 9B). The explanation for this phenomenon can be understood by referring to figure 1 in which it is seen that the upper portion of the anterior left ventricle lies in the proximal zone of VL but in the distal zone of VF while the lower portion of the anterior left ventricle lies in the distal zone of VL, but in the proximal zone of VF. Injury with potassium which tends to involve equal portions of both the proximal and distal zones of both VF and VL will result in an isoelectric ST-segment in both VF and VL since the algebraic summation of the injury potentials derived from the proximal and distal zones of each of these leads will tend to approximate zero. Although no change may occur in ST-VF and ST-VL following such treatment of the anterior left ventricle, the ST-segment in lead VR is displaced downward because the injured area consists chiefly of a part of the distal zone of this lead. Lead I, therefore, exhibits definite ST-segment elevation (fig. 9C, D).

Thus it can be seen that the greater the extent of anterior left ventricular injury the more likely it is that the algebraic summation of injury potentials developed at both the left forelimb and left hindlimb will approximate zero and that no displacement of the ST-segment will occur in leads VF, VL, and III.

b) Heating and cooling the entire anterior left ventricle may also fail to produce any changes in the T-wave in lead III, for reasons similar to those given above for the lack of ST-segment deviation following injury to this region. If the area of the anterior left ventricle treated by heat or cold is of such magnitude that equal portions of the proximal and distal zones of both leads VF and VL are involved, the algebraic summation of the opposite effects which result from treatment of both zones in each lead will tend to approximate zero. The T-wave in both VF and VL would, therefore, tend to remain unaltered, and as a result T-III will remain unchanged. Although T-III remains essentially unchanged following alteration in the rate of repolarization of the anterior left ventricle, the T-wave in lead I shows marked change because the region treated consists chiefly of a portion of the distal zone of lead VR (fig. 15).

SUMMARY AND CONCLUSIONS

The present study of the nature of lead III by analysis of simultaneously recorded 'unipolar' extremity leads VF and VL permits the following conclusions:

1. The five possible combinations of potentials at the left forelimb and the left hindlimb that could account theoretically for upward movement of the beam in lead III and the five possible combinations of potentials at these extremities that could account theoretically for downward movement of the beam in this lead have been

shown to exist experimentally and each combination can be related to the depolarization of a specific ventricular region.

2. It is only when the site of proponderant depolarization lies within the left apex and/or the posterior left ventricle that downward deflection of the beam occurs. in lead III since only depolarization of these segments results in the development of potentials at the left forelimb which are positive relative to those developed at the left hindlimb.   If a portion of this region is activated in advance of the anterior right ventricle, the first deflection of the beam will be downward, inscribing a Q-wave in this lead.   Later excitation of this region will again result in downward deflection of the beam inscribing the downstroke of the R- and S-waves in this lead.

3. It is only when the site of preponderant depolarization lies within the anterior right ventricle and the upper third of the anterior left ventricle that upward deflection of the beam occurs in lead III since only depolarization in these regions gives rise to potentials at the left forelimb which are negative relative to those developed at the left hindlimb.   Early activation of these regions will result in upward deflection of the beam inscribing the upstroke of the Q- and R-waves in this lead.   If a portion of these regions is the last site to become depolarized, the beam will again be deflected upwards, inscribing the upstroke of the S-wave.

4. No deflections of the beam take place in lead III when the major portion of the posterior right ventricle and the mid-third of the anterior left ventricle become depolarized because such depolarization fails to result in the development of any difference in potential between the left forelimb and the left hindlimb.

5. An isopotential ST-segment in lead III can occur when any of the following conditions exist: *a*) no current of injury is present; *b*) injury is present in the mid-third of the posterior right ventricle and/or the mid-third of the anterior left ventricle or injury involves the entire anterior left ventricle; *c*) repolarization has not yet begun in the anterior right and posterior left ventricles; *d*) the anterior right and posterior left ventricles are repolarizing at the same rate, thus producing potentials at the left fore- and hindlimbs of the same magnitude but of opposite electrical sign so that the net potential at each of these extremities is zero.

6. More specific localization of injury to the heart is possible from study of ST-segment displacements in leads VF and VL than from a study of ST-segment displacement in lead III alone.   ST-segment elevation in lead III localizes the injury only to the general region of the left apex or lower two thirds of the posterior left ventricle, while the ST-segment displacements in leads VF and VL help localize the injury to more specific parts of these regions.   ST-segment depression in lead III localizes injury only to the general region of the anterior right ventricle, while the displacements of the ST-segments in leads VF and VL help localize the injury to more specific parts of this region.

7. When the anterior right ventricle repolarizes in advance of the posterior left ventricle, the T-wave is inverted in lead III because of the development of initial relative positivity at the left forelimb.   Conversely, when the posterior left ventricle and left apex repolarize in advance of the anterior right ventricle, the T-wave in lead III is upright because of the development of initial relative negativity at the left forelimb.   The repolarization of the posterior right ventricle and the anterior left

ventricle contributes little or nothing to the configuration of the T-wave in lead III because the repolarization of each of these areas tends to produce simultaneous potentials of the same electrical sign and magnitude at the left fore- and hindlimbs.

These conclusions as to the derivation of lead III in the dog correspond closely with the findings of Hoff, Nahum, and Kaufman (2–5) on the nature of the various components of the ventricular complex in this lead. These authors described lead III as the algebraic summation of potentials derived mainly from the depolarization of the anterior right and posterior left ventricles. They found that depolarization of the anterior left and posterior right ventricles was not represented in lead III. The present study has more exactly delimited the electrically active as well as the electrically neutral areas for lead III, and has established the basis for the presence or absence of electrical representation of the various regions of the heart in lead III.

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RELATIVE DISTRIBUTION OF CARDIAC OUTPUT
IN ACUTE HYPOXEMIA[1]

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THE induction of acute hypoxemia produces a rapid sequence of cardiovascular changes which follow a fairly uniform pattern. It has been shown that the cardiac output and the blood pressure are increased in hypoxemia (1–4). However little information is available on the moment to moment alterations in the circulation of blood during the induction of acute hypoxemia and its readjustment following recovery from the hypoxemic state. Likewise, few data are available on the possible changes in the differential distribution of blood flow during the acute hypoxemic and post-hypoxemic periods. Such data would be valuable in interpreting the hemodynamic changes which occur. In the present study we have attempted to estimate the changes in cardiac output during the various phases of hypoxemia and reoxygenation, and to some extent to determine the relative distribution of the blood flow between the upper and lower parts of the body. This was done by estimating cardiac output on the basis of blood flow through the superior or inferior vena cava.

METHODS

Blood flow was measured in the inferior or superior vena cava with a modified Ludwig stromuhr in vagotomized, heparinized, open-chested dogs, anesthetized with sodium pentobarbital (25 mg/kilo). Flow was determined by the time required for the displacement of 40 cc. volumes in the stromuhr, which required three seconds to 'infinity' (no perceptible flow). The blood flow in the superior vena cava was measured in 7 dogs in 38 periods of hypoxemia and recovery and the flow in the inferior vena cava in 6 dogs in 40 periods. Artificial respiration in open-chested animals assured a controlled respiration throughout the experiment without the intervention of factors related to respiratory failure.

Acute hypoxemia was produced for periods of 2 to 3½ minutes by the substitution of 100 per cent nitrogen for air breathing; reoxygenation was accomplished by returning the animal to air breathing. Serial readings of the blood flow were taken during a control period and repeated each 15 to 30 seconds during the period of hypoxemia and reoxygenation. Mean femoral blood pressures were recorded with a mercury manometer on an ink writing kymograph. The circulation time was estimated in 10 trials on 5 dogs using the acetylcholine method of Wilburne *et al.* (6). This technique measures the time required for circulation of blood from the site of injection to the nodal tissue of the heart, as indicated by a blocked beat.

RESULTS

The changes observed in the hypoxemic and post-hypoxemic periods are illustrated in figure 1 by a graph of typical data obtained in a series of experiments in 2 dogs in which the conditions of anesthesia and blood pressure were similar. Data on the experiments are given in tables 1 and 2.

1. *Effect of acute hypoxemia.* The induction of severe progressive hypoxemia almost always produced a rise in blood pressure ranging from 9 to 44 mm. Hg, which usually began about 30 seconds after the onset of nitrogen breathing. The pressure then leveled off and began to fall rapidly to shock levels. During the first two
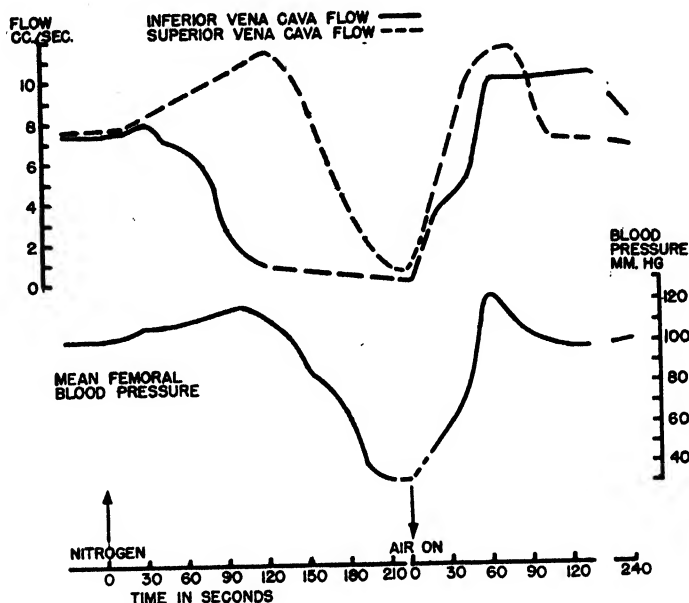
Fig. 1. THE ABOVE GRAPH REPRESENTS the average data obtained during 14 acute hypoxemic and reoxygenation periods in 2 dogs in which conditions of anesthesia and blood pressure were similar. The mean femoral blood pressure shown above is that of the dog in which the superior vena cava flow was measured. The blood pressure changes of the other dog were similar to those shown above.

minutes of the hypoxemic pressor phase a marked acceleration of blood flow up to double the control value was observed in the superior vena cava (fig. 1, table 2).

On the other hand the flow in the inferior vena cava showed only a slight or moderate increase in flow during the early part of this period. Within approximately 75 seconds after the onset of the hypoxemic period, the flow through the inferior vena cava diminished suddenly and markedly although the blood pressure was still above control values. Thirty seconds later no perceptible flow was measurable in this circuit. At this time of markedly diminished flow in the inferior vena cava, the flow through the superior vena cava was usually at or above control values and the blood pressure averaged 87 mm. Hg compared to 93 mm. Hg for the average control value (fig. 1, table 1).

The superior vena cava flow usually (24 of 38 trials) continued at or above the

control flow rates after the blood pressure had fallen below its control level. As the hypoxemia continued, both the blood pressure and superior vena cava flow fell progressively in all instances. In 19 trials the superior vena cava flow stopped almost completely during the late depressor phase of acute hypoxemia, when the blood pressure had fallen to an average of 40 mm Hg.

TABLE 1. FLOW IN THE INFERIOR VENA CAVA

TIME	DOG NO.											
	IVC 1		IVC 2		IVC 3		IVC 4		IVC 5		IVC 6	
	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow
sec.	*mm. Hg*	*cc/min.*	*mm. Hg*	*cc/min.*	*mm. Hg*	*cc/min.*	*mm. Hg*	*cc/min.*	*mm. Hg*	*cc/min.*	*mm. Hg*	*cc/min.*
−5 Nitrogen	77	14.8	101	8.7	75	7.0	108	7.4	94	7.0	94	6.6
0	79	14.3	105	8.7	75	6.7	111	7.4	95	6.7	93	6.1
15	84	15.3	123	9.5	75	7.4	114	7.5	95	6.5	99	7.1
30	94	16.6	147	6.2	79	7.6	130	8.0	101	7.8	103	7.6
45	105	17.4	131	4.2	85	6.7	135	7.0	125	9.9		
60	119	19.0	98	1.3	96	6.0	141	6.6	136	7.1	109	7.6
75	120	17.4	103	0.4	95	6.1	146	5.9	108	1.5	105	7.7
90	108	9.8	*air*	*air*	105	5.0	125	2.7	*air*	*air*	99	6.1
105	84	4.3			98	1.9					90	5.5
120	64	0.5			83	1.1	98	0.7			74	2.5
Air 15	53	·2.8	124	6.3	87	3.6	88	3.4	89	0.7	90	5.2
30									98	5.2	144	9.5
45					151	9.3	129	5.2				
60	127	22.2	115	10.0			156	10	99	7.3	169	11.1
75					165	9.3						
90	112	28.8			134	8.7	140	10	98	8.5	160	10
120	93	25.2			107	8.3	124	10.3	99	8.7	139	8.2
150	90	21.0			90	7.4	122	10	98	8.5	127	7.7
180	86	20.2					117	9.5	96	7.8	115	8.0
240	77	16.7	96	8.0	77	7.1	108	8.0	89	7.3	96	7.4

0 seconds represents the onset of nitrogen breathing. *Air* indicates the time of cessation of nitrogen and the return to air breathing. The data represent average results of several observations on each dog.

When acetylcholine was injected into the femoral vein during the late depressor phase of hypoxemia the average circulation time was greatly increased, averaging 79 seconds in 10 trials as compared to control values averaging 11 seconds. However, this long delay in circulation time was not apparent when the acetylcholine was injected into the heart or into the superior vena cava instead of the femoral vein. The marked slowing of circulation rate in the inferior vena cava and not in the superior vena cava during the depressor phase of acute hypoxemia is in accord with our results obtained with the stromuhr. Direct observation of the heart revealed marked cardiac dilatation, occasional arrhythmias, and slowing during the later stages of the depressor phase of hypoxemia.

2. *Effect of reoxygenation.* With the resumption of air breathing a sudden marked increase in flow through both vena cavae was noted and this was followed almost at once by a rise in blood pressure. In most instances a marked increase in flow was observed even before the blood pressure had risen significantly. Later during this reoxygenation period the blood pressure often reached levels much higher

TABLE 2. FLOW IN THE SUPERIOR VENA CAVA

TIME	SVC 1		SVC 2		SVC 3		SVC 4		SVC 5		SVC 6		SVC 7	
	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow
sec.	mm. Hg	cc/ min.	mm. Hg	cc/ min.	mm. Hg	cc/ min.	mm. Hg	cc/ min.	mm. Hg	cc/ min.	mm. Hg	cc/ min.	mm. Hg	cc/ min.
−5 Nitrogen	82	8.8	112	8.0	88	7.0	80	5.7	64	3.7	88	4.0		7.7
0	82	8.8	108	8.8	84	6.6	82	5.0	64	3.6	88	3.8	102	7.7
15			109	8.0	87	6.3			67	4.4	88	3.4	104	7.7
30	83	10.0	110	10.0			117	6.6	78	5.0			109	8.3
45	85	12.2			95	9.7			74	5.7	116	7.3	108	8.7
60	90	13.2	112	10.0	98	10.0	106	2.7	86	6.8	118	10.0	110	9.3
75	91	16.0	113	13.3	102	10.8			103	10.0	118	16.0		
90	75	11.0	108	13.3	98	11.4	55	0.9	88	8.0	101	13.3	117	10.3
105	52	6.9	100	13.3	83	9.9			87	8.0	89	13.3	118	11.4
120	40	2.7	82	11.4	63	5.3	air	air	72	4.0	70	11.4	112	12.5
135	35	1.1	68	8.0					42	1.3	60	8.0	106	12.9
150	air	air	51	5.0	44	2.6			43	0	51	7.3	88	9.3
165			28	0	42	2.5			air	air	47	5.7	78	6.3
180			air	air	39	1.7					48	5.0	67	4.4
195					air	air					50	4.4	39	2.2
210											air	air	33	0.9
Air														
15					67	4.4					58	6.6	45	3.6
30							88	5.7						
45					120	11.4					110	10.0	81	10.0
60	73	12.5	45	10.0									123	11.7
75			110	13.3					47	4.0	118	7.3		
90	101	14.3	140	10.0	99	8.3	72	7.3					105	7.0
120			170	13.3					83	5.0	121	7.3	96	7.0
240	77	8.4	120	8.0		7.1		5.7	60	3.8		5.7		6.6

See footnote to table 1.

than in the early hypoxemic phase. The pressor effect was soon dissipated and the blood pressure and blood flow through both vena cavae returned to control levels approximately four minutes after resumption of air breathing.

DISCUSSION

These results suggest that the first effect of hypoxemia was to produce an increase in the rate of return blood flow to the heart and thus an increase in the cardiac output. This was accompanied by a rise in blood pressure. The augmented blood flow

would act to increase the perfusion of the tissues and would normally compensate for the hypoxemia. As the hypoxemia continued, the flow in the inferior vena cava began to fall and was markedly reduced even though the blood pressure and superior vena cava flow were still above control values. It is apparent that at this time, a preferential redistribution of blood to the vital head region occurs. Continuance of the hypoxemia results in a fall in the superior vena cava flow as well as in the inferior vena cava flow, and this is furthered by progressive cardiac failure.

It is possible that our mode of flow measurement does not give an entirely satisfactory picture of the cardiac output, since we are measuring the venous return to the heart exclusive of the azygos flow and the important coronary flow. The pooling of blood in the periphery as a result of vasodilatation would unquestionably introduce deviations which we could not determine with our technique. Nevertheless our technique does offer an index of the cardiac output which appears to be in accord with our findings on the changes in circulation time during hypoxia. The flow of blood through the coronary circuit which we could not measure, appears to play an important rôle in the mechanism of recovery.

With reoxygenation the blood flow increases almost at once and this is quickly followed by a marked rise in arterial pressure. These changes are made possible by rapid recovery of the heart as the result of an adequate oxygen supply, as well as by vasoconstriction in the periphery. At this time endogenous pressor materials produced during the hypoxemic period probably contribute to the blood pressure rise (6).

Our experiments suggest that some of the blood pressure effects seen in acute hypoxemia and in the reoxygenation phase are related to the marked changes in cardiac output. These output changes are due most likely to vasodilating effects induced by tissue hypoxia upon the peripheral vessels. As a consequence a redistribution of the circulating blood flow occurs. There can be little doubt that central nervous mechanisms as well as humoral factors contribute to these effects. Our results are in accord with other findings that the cardiac output is a function of the tissue oxygen requirements and that when tissue hypoxia exists the cardiac output will rise as a compensatory mechanism. When the lack of oxygen becomes extreme the heart and central nervous system begin to weaken and the cardiac output falls probably because of heart failure. With resaturation of the blood and tissues with oxygen, the chain of events is reversed and cardiac output rapidly returns to its basal level with a transitory overswing.

With our technique we have been able to perfuse tissues with hypoxemic blood without a concomitant hypercapnia. Consequently, the large changes in blood flow occurring under these conditions are not explained by alterations in CO_2 content. Reoxygenation of the blood results in an increased flow similar to that seen in local reactive hyperemia first described by Lewis (7). Thus it would appear that a large part of the stimulus for this reactive hyperemia throughout the body must be due directly to oxygen lack and not entirely to the production during the hypoxemic period of other easily diffusible substances which can be lost in the lungs or quickly disposed of by the liver.

This is indicated by the remarkably rapid recovery of the severely hypoxemic

heart upon resumption of air breathing.   During the late phases of the hypoxemic period, the cardiac output as measured by the flow in both venae cavae was extremely low.   The heart could be seen to be dilated and beating feebly.   Nevertheless, within a few seconds after the reestablishment of air breathing the heart was seen to beat vigorously again, cardiac output markedly improved and the blood pressure rose rapidly.   The fact that oxygenated blood from the lungs is able to reach the coronary circulation so rapidly and in sufficient amounts to effect such a rapid recovery, indicates that some residual flow must have been continuing through the heart-lung-coronary circulation.   Such blood would include that being returned through the coronary vessels and perhaps some via the azygous veins.   The flowmeter offers some resistance to flow and if only a small amount of blood were being pumped by the heart this blood could easily bypass the flowmeter and pass through non-cannulated parallel circuits which offer less resistance.

SUMMARY

The return of blood flow to the heart through the superior or the inferior vena cava was measured in 13 dogs subjected to acute hypoxia produced by nitrogen breathing.   With the onset of acute hypoxemia the blood flow and pressure increased. After about 75 seconds the flow through the inferior vena cava fell rapidly to nearly zero levels although the superior vena cava flow remained above normal.   As the hypoxemia progressed, the superior vena cava flow also diminished.   At this time the blood pressure was low and the heart was seen to be dilated and beating slowly. Reoxygenation resulted in an immediate recovery of cardiac function of blood flow in both circuits and of the blood pressure which returned to the previous control levels within a few minutes with a temporary overswing.

The mechanisms of some of the hemodynamic changes occurring during the induction of and recovery from acute hypoxemia are discussed.

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NATURE OF THE TWO PRESSOR RESPONSES TO ACUTE HYPOXEMIA WITH SOME OBSERVATIONS ON THE RÔLE OF THE ADRENALS IN HYPOXIA[1]

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WE HAVE described, in detail, the responses of the blood pressure and heart rate to acute severe hypoxemia (1). During such a hypoxemia, produced in open-chested dogs by breathing pure nitrogen, the rise in pressure (hypoxemic pressor phase) is followed by a fall (hypoxemic depressor phase). The depressor phase is progressive ending in death if nitrogen breathing is maintained. However, when air is substituted for nitrogen, a second pressor effect (post-hypoxemic pressor phase) is seen which is frequently greater in magnitude than the primary pressor effect.

We have shown that there is a relationship between the height of the post-hypoxemic rise and the duration of the hypoxemic depressor phase. This relationship suggests that, during the depressor phase, substances were elaborated which could not exert their pressor effect or be quickly destroyed in the absence of oxygen. With reoxygenation, the pressor action of these substances becomes manifest. This concept was strengthened by our finding that injected epinephrine acts similarly, with no pressor effect during the late hypoxemic depressor phase and a pressor action as soon as air breathing is resumed. This led us to conclude that the pressor material liberated during the hypoxemic phase was epinephrine-like in action. The nature of the hypoxemic pressor phase was not indicated by our experiments. The present study was undertaken to test these concepts and to define the rôle of the adrenal gland in these pressor response to acute hypoxemia.

METHODS

Eighty-three experiments were performed upon 12 open-chested vagotomized dogs, anesthetized with pentobarbital sodium (25 mg/kg.). The thorax was opened in the fifth or sixth intercostal space, the adjacent ribs were maintained widely retracted and the thin mediastinal septum was destroyed. Hypoxemia was produced by replacing the respired air with nitrogen (1).

Six of the 12 animals were adrenalectomized and the other 6 were prepared by a modification of the technique described by Bouckaert and Van Loo (2). Our technique involves elimination of the right adrenal gland from the circulation by

ligature and the isolation of the venous drainage from the intact innervated left adrenal gland. All veins tributary to the left adrenal gland drainage were tied off, leaving undisturbed only the two major venous trunks, the adreno-caval and the lumbo-adrenal veins. The former was maintained intact, being cleared from the surrounding tissue so that it could be clamped as desired. The lumbo-adrenal vein was cannulated a short distance below the point where it entered the capsule of the adrenal gland, with the cannula opening toward the hilus. The cannula was kept open by means of a slow saline drip under constant pressure. During this time, the venous output of the left adrenal gland drained, via the adreno-caval vein, into the inferior vena cava and thus was in the circulation of the animal. When it was desired to eliminate the effects of the adrenal from the experiment, the adreno-caval vein was clamped and gentle suction was applied by means of a syringe attached to the cannula via a three-way stopcock and the experiment was repeated. In this way the entire venous outflow from the left adrenal gland could be collected and measured, while the responses of the animal with the adrenal out of the circulation were being recorded. To test the pressor effect of the blood so collected upon the same animal, the clamp on the adreno-caval vein was removed and the collected blood was reinjected within about 10 secs. Blood pressures were recorded continuously upon a kymograph with a mercury manometer.

RESULTS

1. *Hypoxemic Pressor Phase.* The pressor response during the hypoxemic period is usually definitely reduced by the diversion of adrenal blood from the circulation. This is clearly illustrated in figure 1. While this relationship does not appear in every instance and at times the responses are similar with the gland in and out of the circulation, it is revealed by a comparison of the averages of the absolute rises in pressure seen in two types of experiments. These figures, given in table 1, show that on the average the absolute level attained is only slightly higher when the adrenal secretion participates in the response. Thus the average response in the intact animal was a rise of 33 mm. Hg, while the average response in the animal with the adrenal out of the circulation was 27 mm. Hg. Experiments with the adrenal in and out of the circulation were conducted alternatively to compensate for changes in the control blood pressure level and general condition of the dog as the experiment proceeded. The time at which the hypoxemic peak was attained was the same in both instances.

2. *Changes Occurring After the Reinstitution of Air Breathing.* We have compared the post-hypoxemic pressure maxima obtained in experiments in which the adrenal venous drainage was alternatively permitted to flow into the systemic blood stream and, on the next trial, diverted into a syringe. As can be seen from a typical experiment in figure 1, there is a marked difference in the post-hypoxemic maxima obtained under these two conditions. In figure 1A, it will be seen that when the adrenal was participating, the resumption of air breathing was followed by a pressure increase of 54 mm. Hg above the reference level. In figure 1B, the experiment was repeated with the adrenal drainage diverted from the circulation and being collected in a syringe. Following resumption of air breathing in this case the pressure returned

to approximately the reference level.   After the pressure had leveled off close to the control blood pressure level, the collected blood was reinjected into the blood stream. A second rise was thus produced with the blood pressure level now reaching an aver-
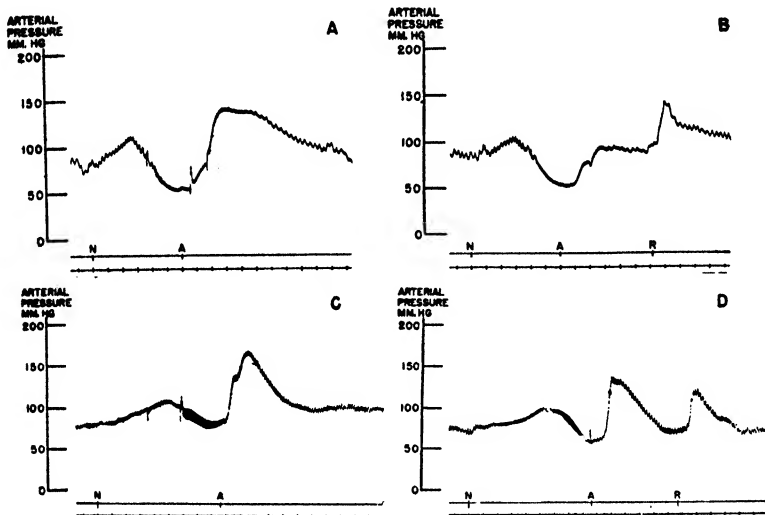
Fig. 1. BLOOD PRESSURE IN FEMORAL ARTERY.   Time in 15-sec. intervals.   *N* indicates time of onset of nitrogen breathing and *A*, onset of reinstitution of air-breathing.   Segments A and B are from *dog 1;* C and D from *dog 3*.   Segments A and C serve as controls, the adrenal blood is draining into the systemic circulation.   In segments B and D, the adrenal venous blood is diverted from the circulation beginning with the onset of nitrogen breathing and is reinjected into the systemic circulation at *R*.   Discussed in text.

TABLE 1.   ARTERIAL PRESSURE RESPONSE DURING NITROGEN BREATHING

DOG NO.	ADRENAL GLAND IN CIRCULATION			ADRENAL GLAND NOT IN CIRCULATION		
	No. of exper.	Av. max. blood pressure level	Av. blood pressure rise above reference level	No. of exper.	Av. max. blood pressure level	Av. blood pressure rise above reference level
		mm. Hg	*mm. Hg*		*mm. Hg*	*mm. Hg*
1	6	120	25	4	111	11
2	11	89	27	7	77	34
3	2	127	29	2	104	16
4	4	126	30	4	114	23
5	2	135	48	2	115	32
6	5	130	54	5	114	39

age of 54 mm. Hg above the reference level.   Thus, the rôle of the adrenal secretion in the production of the post-hypoxemic rise is clearly portrayed.

Data in table 2 compare the rise in arterial pressure seen in other alternate experiments with the adrenal gland in and out of the circulation.   Elimination of the adrenal gland from the circulation is seen to result in a markedly diminished rise in the arterial pressure after the reinstitution of air breathing.   Thus the average

TABLE 2. ARTERIAL PRESSURE RESPONSE ON RESUMING AIR BREATHING FOLLOWING NITROGEN BREATHING

DOG NO.	N₂ BREATHING	ADRENAL IN CIRCULATION		ADRENAL OUT OF CIRCULATION		
		Control blood pressure	Rise above reference level	Control blood pressure	Rise above reference level	Pressor activity of blood
	sec.	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1	80	104	12	98	0	—
	75	104	12	104	−12	20
	90	90	54	88	+2	52
	90	72	64	88	0	38
2	155	70	70	48	54	50
	155	56	30	44	30	32
	205	56	44	50	46	44
	205	36	73	36	18	22
	240	42	50	42	22	12
3	120	114	98	98	46	43
	130	82	88	80	58	48
4	150	92	86	92	24	48
	120	112	16	98	6	32
	120	94	20	92	20	28
	120	84	38	84	24	16
5	180	80	114	62	60	66
	180	94	58	94	42	46
6	120	92	110	74	42	42
	120	80	42	80	−12	30
	120	74	2	78	−16	20
	120	66	−6	72	−16	32
	120	70	−16	72	−28	12

TABLE 3. RELATION OF DURATION OF HYPOXEMIC PERIOD TO BLOOD PRESSURE RESPONSE IN A TYPICAL BILATERALLY ADRENALECTOMIZED DOG

PER. OF N₂ BREATHING	CONTROL BLOOD PRESSURE	HYPOXEMIC PEAK	HYPOXEMIC RISE[1]	POST-HYPOXEMIC PEAK	POST-HYPOXEMIC RISE[1]
sec.	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
90	82	104	22	70	−12
120	102	116	14	88	14
150	84	104	20	126	42
180	86	104	18	144	58
210	84	106	22	158	74
240	78	102	24	158	80
300[2]	70	102	32	78	8

[1] Above the reference level.   [2] This trial was followed by death of the animal.

response in the intact animal was a rise of 46 mm. Hg, while in the animal with the adrenals out of the circulation the rise is only 23 mm. Hg. The reinjection of the collected blood caused an average rise of 33 mm. Hg, more than enough to account

for the deficit in the physiologically adrenalectomized animal. However, that extra-adrenal factors may also operate by themselves to produce a post-hypoxemic rise above the reference level is shown in table 3.

The 6 animals which were bilaterally adrenalectomized were subjected to varying periods of nitrogen breathing. The responses of the arterial pressure in the hypoxemic and post-hypoxemic periods in a representative example are tabulated in table 3. It will be seen that both the absolute level of the post-hypoxemic rise and the rise as measured from the reference level appear to be related directly to the duration of the hypoxemic period.

3. *Pressor Activity of the Blood Collected During Hypoxemia.* After the post-hypoxemic peak due to extra-adrenal mechanisms was recorded, we determined the pressor activity of the blood which had been collected during the hypoxemic period. This was done in two ways in different experiments: *a*) the blood was reinjected into the animal immediately after the initial post-hypoxemic peak was reached and the response noted (fig. 1B) and *b*) the blood was reinjected at a later period as the blood pressure reached the control levels (fig. 1D). Data on pressor activity are given in table 2. The correspondence between the average absolute pressures attained during the post-hypoxemic phase when the adrenal gland was in the circulation, and the average totals obtained by adding the pressor activity of the blood to the extra-adrenal post-hypoxemic peaks is apparent.

In order to ascertain when the greatest amount of pressor activity appeared in the blood of the adrenal drainage, during the period of hypoxemia, the blood in 10 experiments was collected in several samples, each of which was later separately tested for its pressor activity. The rate of blood flow from the adrenal in cc/min. was also noted in order that some conclusions regarding the concentration of pressor material might be drawn. Our results show clearly that, during the period when the systemic pressure is elevated, there is a definite increase in venous drainage from the adrenal gland. Values for adrenal flow during control periods ranged from 1.5 to 4 cc/min. During the elevation of blood pressure in the hypoxemic phase the measured flow increased from 50 to 100 per cent. It was also clear that while the volume of the blood collected during the hypoxemic pressor phase was much greater than that obtained during the hypoxemic depressor phase, the pressor activity of the former blood was markedly less than that of the smaller volume obtained during the later depressor stages of nitrogen breathing. For example, during a typical experiment in which nitrogen breathing was continued for four minutes, three blood samples were collected. The first, containing 7.5 cc., was collected during the first 2 minutes (hypoxemic pressor phase), the second, containing 3.5 cc., during the next 1½ minutes (hypoxemic depressor phase) and the third, containing 2 cc., was obtained during the 50 seconds following the reinstitution of air breathing while the arterial pressure was at its lowest point during the experiment (the blood drainage from the adrenal gland at this time being still markedly hypoxemic as indicated by its color). When these samples were reinjected into the animal at approximately control blood pressure levels, the first sample produced a rise of 2 mm. Hg, the second, a rise of 14 mm. Hg and the third and smallest sample, a rise of 22 mm. Hg. Thus it is clear that the greatest production of pressor material by the adrenal gland occurs during the later stages of hypoxemia while the blood pressure is falling. It also

appears that adrenal drainage collected during the hypoxemic pressor phase shows no greater pressor activity than blood collected during control periods of air breathing.

In order to obtain further information regarding the pressor activity of adrenal drainage and its relation to adrenal flow and systemic arterial pressure, we collected the adrenal outflow in six experiments during a period of marked elevation of the arterial pressure produced by intravenous injection of 0.2 mg. epinephrine while the animal continued to breathe air. During a representative experiment, the pressure rose from a level of 40 mm. Hg to a level of 184 mm. Hg. The adrenal blood collected during the 265 seconds after injection of epinephrine totaled 14 cc. When reinjected into the animal after its return to control pressure levels, this blood produced a rise

TABLE 4. PRESSOR ACTIVITY OF ADRENAL VENOUS DRAINAGE DURING AIR BREATHING AND CHANGES IN SYSTEMIC ARTERIAL PRESSURE DURING COLLECTING PERIOD

DOG NO.	COLLECTION PER.	BLOOD PRESSURE AT BEGINNING OF COLLECTION PER.	BLOOD PRESSURE AT END OF COLLECTION PER.	AMOUNT OF BLOOD COLLECTED	BLOOD PRESSURE ATTAINED WITH REINJECTION OF COLLECTED BLOOD	PRESSOR ACTIVITY
	sec.	mm. Hg	mm. Hg	cc.	mm. Hg	mm. Hg
1	150	82	64	9	76	12
2	150	84	60	8	70	10
	155	43	32	8½	46	14
	205	40	32	8	42	10
	245	46	34	6	44	10
	660	40	26	9½	38	12
3	150	82	74	4½	84	10
	150	60	50	3	54	4
	165	88	82	7	96	14
4	120	98	86	5	96	10
5	180	144	108	9	144	36
	180	88	50	7	96	46
6	120	84	76	11	92	16
	120	78	70	10	82	12

of 2 mm. Hg. Collection of adrenal blood for an equal period of time, in this animal, before epinephrine was given yielded a volume of 6 cc. which produced a rise of 10 mm. Hg. It appears from these results that there is a decrease of pressor activity during the time of epinephrine action as compared with the controls, but apparently no primary relation between the pressor activity of adrenal drainage and its volume.

4. *Pressor Activity of the Adrenal Venous Drainage During Control Periods of Air Breathing.* Blood was collected from the adrenal gland in 14 experiments for varying periods of time after the blood pressure had become stabilized and while the animal was lying quietly during air breathing. In each of 6 animals there was a slow but steady fall averaging 14 mm. Hg while the adrenal, blood was being collected. At the conclusion of the collection period, this blood was reinjected into the animal. In each of the 6 animals there was a slow, but steady fall in systemic

arterial pressure while adrenal blood was being collected. In each instance, reinjection caused a rise of arterial pressure to a level closely approximating that existing at the beginning of the collection period. Data on this point are given in table 4.

<div align="center">DISCUSSION</div>

It has been known for some time than an animal made acutely hypoxemic will respond with a rise in the arterial pressure which, with continuing hypoxemia, then falls until the animal succumbs. If air breathing is reinstituted after hypoxemia, a post-hypoxemic rise in pressure is produced which often exceeds that of the hypoxemic elevation. The adrenal gland, the heart and the sympathetic system have been implicated in these responses. Kaya and Starling (3) maintained that the post-hypoxemic pressure augmentation resulted from an increase in contractile power of the heart caused by oxygen. Mathison (4) mentions an effect of oxygen upon the vasomotor center. Other authors (5) have maintained that there is a post-hypoxemic excitation of the sympathetic nervous system. Stavraky (6) has reported that adrenalectomized animals on optimal ventilation show a hypoxemic rise with nitrogen breathing and also a prominent post-hypoxemic rise after oxygen breathing is resumed. As we have commented previously (1) there has been no reported quantitation of the rôle of the adrenal gland in these changes as compared with adequate controls in the same animal. On the basis of a relationship demonstrated between the hypoxemic depressor phase and the height of the post-hypoxemic rise, it was presumed that an epinephrine-like pressor material was liberated during acute hypoxemia (1). This concept is in agreement with other reported evidence (10). It was also demonstrated that epinephrine injected intravenously, during the depressor phase of acute hypoxemia, produced little or no pressor response. The data we have presented in this report demonstrate that the adrenal gland plays only a minor rôle in the production of the rise in arterial pressure during the phase of nitrogen breathing. Its rôle, however, in the production of the post-hypoxemic rise, while variable, is a major one. It is clear that the extra-adrenal factors, probably involving the sympathetics, are in themselves capable of producing a post-hypoxemic rise which may go above the level of the control pressure, but in the absence of the venous drainage from the adrenal gland, the post-hypoxemic rise is considerably diminished. However, even in its diminished form, the magnitude of the post-hypoxemic rise resulting from extra-adrenal factors is itself a function of the duration of the hypoxemic period. These data confirm our previous conclusions that in the absence of oxygen the pressor material liberated in the body during hypoxemia is powerless to act. Only as oxygen again reaches the tissues is its pressor effect revealed.

Our data also demonstrate that the greatest production of pressor material by the adrenal gland occurs in late hypoxemia when the blood pressure is falling. It is not significantly increased during the period of the hypoxemic rise (10). Since we found that the withdrawal of adrenal drainage in air-breathing leads to a drop in blood pressure, the differences seen in the hypoxemic rises with the adrenal in and out of the circulation, may be due, at least partially, to the same loss (compare table 1 with table 4). The rate of flow of adrenal venous drainage maintains a direct relationship to the level of the arterial pressure, but there is no relationship of the

pressor activity of the collected blood to the rate of flow (10). In fact, it appears that while the rate of venous drainage from the adrenal gland is high during a period of elevation of the systemic blood pressure caused by the injection of epinephrine during air breathing, the pressor activity of this blood is, if anything, lower than that collected during control periods of air-breathing. The mechanism of the apparent reduction in adrenal activity, during the pressor phase of epinephrine, is probably related either to a diminution of the sympathetic tone or to a humoral inhibition of the gland caused by circulating injected epinephrine or, to both mechanisms (11).

Our results also indicate that removal of the venous drainage of the adrenal gland from the systemic circulation is accompanied by a slow steady fall in the arterial pressure and that reinjection of the collected blood raises the arterial pressure approximately to the level of the systemic arterial pressure when the collection was begun. Thus, at least under the conditions of our experiments the adrenal appears to play a significant rôle in the regulation of the blood pressure (cf. 7 and 8). Our evidence that hypoxemia in anesthetized animals causes an increased liberation of pressor material from adrenals seemingly is opposed to other published results (9).

It is justified to conclude that the adrenals come into play in conditions of low blood pressure, as in shock and in hypoxemia, to help neutralize the ill effects of these states by raising the blood pressure and redistributing blood to vital organs.

SUMMARY

The factors responsible for the pressor responses during the hypoxemic phase and in the post-hypoxemic phase were investigated. In unilaterally adrenalectomized animals, diversion into a syringe of the venous blood draining from the remaining adrenal resulted in no significant change in the hypoxemic pressor phase, but diminished considerably the post-hypoxemic response. Blood collected in the phase of the falling blood pressure during nitrogen breathing had markedly more pressor activity than that collected during the earlier phases of hypoxemia. Reinjection of such collected blood immediately after the peak of the post-hypoxemic response due to extra-adrenal factors caused the pressure to rise to a level similar to that attained in the control experiment.

It is concluded that the adrenal gland plays little part in the production of the hypoxemic pressor response, but plays a major rôle in the production of the arterial pressor response after re-aeration. Pressor material liberated from the adrenal during severe hypoxemia does not exert pressor effect until tissues are reoxygenated.

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LUNG FUNCTION STUDIES. II. THE RESPIRATORY DEAD SPACE[1,2]

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CERTAIN methods of estimating pulmonary ventilatory efficiency are limited by the accuracy of the measurement of respiratory dead space (1, 2). Krogh and Lindhard measured the physiological dead space and concluded that it varied within narrow limits during changes in lung inflation (3); on the other hand, Haldane and Priestley (4) maintained that the dead space might increase as much as 800 cc. during maximal lung inflation. Though subsequent investigations (5, 6) have in general confirmed the work of Krogh and Lindhard, there still remains considerable uncertainty about the magnitude and constancy of the volume of the respiratory dead space. Within the past few years some investigators have employed a single dead space volume for different tidal volumes (1) whereas others have used two values for dead space, one for shallow and another for deeper breaths (7). The development by Lilly and Hervey (8) of the nitrogen meter, for continuous analysis of the nitrogen concentration of respired gases, has made possible the reinvestigation of this problem.

Since the terminology used by various writers is not uniform, it seems advisable to clarify the meaning of dead space. The respiratory system may be divided into those parts which serve primarily as a conducting airway and not as sites for rapid change of O_2 and CO_2 (mouth, nose, pharynx, larynx, trachea, bronchi and bronchioles) and those whose chief function is gas exchange (alveoli, alveolar sacs and atria). If a sharp separation could be made between the two, the former would be defined as the dead space and the latter as the container of alveolar air. If the dead space gas could be expelled from the respiratory tract as a bloc of gas with a sharp boundary line dividing it from the alveolar gas, its measurement would be simplified. However several factors prevent this: first, anatomical studies indicate that the boundary between conducting and exchange airway is not definite; second, diffusion occurs at this boundary area and obscures any sharp margin; third, when the gas is put into motion during expiration, some alveolar gas pushes into the dead space gas and so eliminates a square front. The latter process of expiratory gas mixing

in the conducting airway must be subject to aerodynamic variables which are independent of the original separation of dead space gas from alveolar gas.

However it has been found possible, by simultaneous measurement of nitrogen concentration and volume flow of expired gas, to separate dead space gas from alveolar gas by an application of Bohr's formula. Throughout this paper, the term 'physiological dead space' will be used to refer to the volume of the conducting airway down to the location at which a large change in gas composition occurs. This measurement of physiological dead space will change not only with alterations of the caliber of the airway, but also with the extent of boundary diffusion between the alveolar and dead space gases; the latter, among other factors, is a function of time and hence of the rate of respiration.

The effective ventilation of the residual air by a given tidal volume is reduced by the physiological dead space and also by non-uniform ventilation of the residual air. Therefore ineffective tidal volume has these two components; it is believed that the method to be described may differentiate the two.

METHODS

This method is based upon the continuous and simultaneous measurement of *a*) expired gas volume-flow and *b*) expired gas N_2 content, following the change from breathing air to breathing 99.6 per cent oxygen.

1. N_2 Analysis. The nitrogen meter developed by Lilly and Hervey (8) continuously samples (using about 1.0 cc/sec.), analyzes and records the nitrogen content of constant composition samples with an accuracy from day to day of $\pm$ 2 per cent N_2, relative to Haldane analyses. This is an overall accuracy summing errors in sampling methods, measurement of records and setting the instrument. In our instrument the response to a step-wise change in gas composition occurs with a delay of 0.03 to 0.05 second between the time the sample enters the instrument and the time at which the record starts to change; 95 per cent of the final response is attained within an additional 0.03 second and maximal response is attained 0.10 to 0.12 second after the sample enters the instrument. The delay in the final 5% of response may not be instrumental, but due to our inability to produce a completely square gas front.

2. Volume-flow Analysis. Expired gas is passed through a flow meter, which continuously measures the pressure differential across a 400-mesh screen in the flow path, by means of an electrical capacitance manometer (9). Photographic recording of N_2 concentration and flow is made by appropriate oscillograph galvanometer-camera systems. With the flow meter connected as described below, the response to a step-wise change in flow occurs with a starting delay in recording of not more than 0.03 second and maximal response is attained in an additional 0.05 to 0.07 second. The flow meter response is calibrated daily by blowing compressed air through a rotameter to it. Volumes presented hereafter are derived without temperature correction from the rotameter calibrated at 760 mm. Hg and 21.1°C. The area of the flow tracing, as it is deflected from the zero line by an expired breath, can be converted to a volume measurement by measurement with a planimeter or with a squared transparent sheet. The error in volume measurements by this method is

±5 per cent; this combines errors in rotameter (±2%), flow meter calibration and record measurement.   In tables 1, 2, 3, 4 and 6, the figures for tidal volume represent the average of three successive expirations.   Since the various patterns of breathing were maintained for at least 8 to 10 breaths, it is reasonable to assume that the expiratory volumes were not greatly different from inspiratory or tidal volume.

TABLE 1.   PHYSICAL AND REPIRATORY DATA ON 45 MALE AND 4 FEMALE SUBJECTS

	AGE	HEIGHT	WEIGHT	EXP. VOL. TO WASH OUT D.S.[1]	PHYSIOL. DEAD SPACE[1]	TIDAL VOL.[1]	$\frac{P.D.S.}{T.V.} \times 100$	RESP/MIN.
				45 Male Subjects				
	yr.	in.	lb.	cc.	cc.	cc.		
Mean.............	26.4	70.8	161.5	325	156	651	25.9	15.6
S.E. mean (±)......	0.9	0.4	3.3	10	4	33	1.1	0.6
S.D. (±)..........	5.7	2.5	22.3	65	28	222	7.6	4.1
Coeff. variation.....	22%	3.5%	14%	20%	18%	34%	29%	26%
Range............	19–38	65–77	120–210	207–472	106–219	276–1448	10–43	8.5–27
				4 Female Subjects				
Mean.............	24	63.5	120	250	104	589	20	15.5

[1] Average, first three breaths.

TABLE 2.   EFFECT OF VOLUNTARY HYPERVENTILATION ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	QUIET BREATHING		VOLUNTARY HYPERVENTILATION	
	Tidal vol.	Physiol. D. S.	Tidal vol.	Physiol. D. S.
	cc.	cc.	cc.	cc.
20	636	111	3085	167
21	478	157	2500	203
22	476	202	1410	253
23	628	147	2105	242
24	683	179	1540	261

TABLE 3.   PHYSIOLOGICAL DEAD SPACE IN VOLUNTARY AND EXERCISE HYPERPNEA

	SUBJ. NO. 34 (MALE)			SUBJ. NO. 48 (FEMALE)		
	Tidal vol.	Physiol. D.S.	Insp. time	Tidal vol.	Physiol. D.S.	Insp. Time
	cc.	cc.	sec.	cc.	cc.	sec.
Voluntary hyperventilation......	910	186	1.1	1200	170	0.9
Post-exercise hyperpnea.........	810	188	1.0	1310	158	1.3
Quiet breathing................	580	168	1.7	895	105	2.5

3. Other Apparatus.   A nose clip and rubber mouthpiece of 2 cm. internal diameter were used.   The mouthpiece was connected to a four-way metal valve of 2.1 cm. internal diameter.   Instrumental dead spaces were respectively 40 cc. and 60 cc. when room air or oxygen was breathed.   Oxygen (99.6% ± 0.1%) was delivered from a high pressure tank through a demand valve designed to operate with

TABLE 4. EFFECT OF END-INSPIRATORY LUNG VOLUME ON PHYSIOLOGICAL DEAD SPACE

START OF INSPIRATION AT	SUBJ. NO. 34 (MALE)			SUBJ. NO. 48 (FEMALE)		
	Tidal vol.	Physiol. D.S.	Insp. time	Tidal vol.	Physiol. D.S.	Insp. time
	cc.	*cc.*	*sec.*	*cc.*	*cc.*	*sec.*
Max. exp. pos..................	440	119	1.3	660	84	2.2
Normal exp. pos...............	580	168	1.7	895	105	2.5
High insp. pos................	650	233	1.2	830	202	1.1

TABLE 5. EFFECT OF BREATHHOLDING ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	QUIET BREATHING			BREATHHOLDING			
	Exp. No.	Insp. time	Physiol. D.S.	Exp. no.	Insp. time	Physiol. D.S.	Diff. of mean D.S.
		sec.	*cc.*		*sec.*	*cc.*	
50	1	2.0	132	2	20	70	
	3	2.0	128	4	21	66	62
51	1	1.3	160	2	20	95	
	3	1.3	173	4	21	74	82
34	1	2.0	168	2	22	107	61
48	1	2.5	107	2	21	56	
	3	2.2	114	4	20	61	52
52	1	2.5	192	2	22	136	
	3	2.1	178	4	21	145	44

TABLE 6. EFFECT OF SMALL INCREASE OF INSPIRATORY TIME ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	TYPE OF RESPIRATION	PHYSIOL. D.S.	TIDAL VOL.	INSP. TIME	PHYSIOL. D.S. a-b	TIME b-a
		cc.	*cc.*	*cc.*		
53	a. Fast insp. & exp.	218	1350	1.3		
	b. Slow insp. & exp.	135	1350	5.2	83	3.9
54	a. Fast insp. & exp.	228	770	1.0		
	b. Slow insp. & exp.	178	1060	2.5	50	1.5
55	a. Fast insp. & exp.	229	1130	1.4		
	b. Slow insp. & exp.	159	1160	2.9	70	1.5
34	a. Regular rhythm	172	572	1.1		
	b. Short insp. pause	116	544	3.8	61	2.7
48	a. Regular rhythm	100	765	1.9		
	b. Short insp. pause	76	740	3.8	24	1.9

low inspiratory resistance. Expired gas was conducted through the four-way valve, a Sadd valve and 30 inches of flexible rubber tubing with 2 cm. internal diameter to the flow meter.

The sampling needle of the nitrogen meter was inserted into the middle of the mouthpiece lumen just external to the subject's lips, adding an apparatus *expiratory* dead space of 2 to 3 cc. Since measurements of physiological dead space were made during expiration, no apparatus correction was made.

Subjects sat quietly in a chair and breathed room air through the mouthpiece for several minutes. The oxygen system was flushed and, during an expiration, the room air orifice was closed so that oxygen was breathed on the following inspiration and thereafter.

4. Subjects. The subjects were healthy white males and females between the ages of 19 and 38 years. Certain physical characteristics are given in table 1.

5. Analysis of Records. Figure 1 shows the type of record obtained when oxygen is breathed after breathing room air. During inspiration, oxygen (0.4% N_2) is inhaled. The expired gas may be divided into three nitrogen-fraction phases (8): the first part, approximately 20 to 100 cc. of oxygen and water vapor, represents inspired gas remaining in the upper respiratory tract; a final portion with relatively constant N_2 content probably represents 'alveolar' gas; a mid-portion of about 100 to 300 cc. of gas with a rapidly rising N_2 content represents a mixture of pure inspired gas and alveolar gas, the mixing presumably being accomplished by boundary diffusion and by expiratory flow conditions in the upper airway.

From the nitrogen and flow curves one can obtain the data to solve Bohr's formula for dead space:

$V_e \times C_e = (V_e - V_{ds}) C_a + V_{ds} \times C_i$ in which

V_e = Volume of expired air.

C_e = Concentration of a gas in V_e.

V_{ds} = Volume of the dead space.

C_a = Concentration of the same gas in alveolar air.

C_i = Concentration of the same gas in inspired air.

This states that an expired breath consists of a mixture of two parts, each with a definite concentration of a given gas. V_e, C_i (approximately zero for N_2) and C_a (the N_2 concentration of the alveolar phase) may be measured directly from the record. C_e is obtained by correcting the N_2 curve for flow variations and measuring the area under it, thus obtaining volume of N_2 expired; this is divided by total volume expired, V_e, to give C_e. The equation may then be solved for dead space. Measurements made in this way will be called 'calculated' physiological dead space.

The factor of non-uniformity of alveolar gas is eliminated as follows: The O_2 remaining in the conducting airway after inspiration is mixed in the airway on expiration with alveolar gas similar to that expired immediately after the dead space has been washed out. The N_2 concentration of this alveolar gas is measured and inserted in Bohr's formula. Similarly V_e is taken as the volume expired up to the point at which this initial alveolar concentration is reached. If the N_2 content of alveolar gas is uniform, i.e. the alveolar plateau is flat, the same result is obtained if

any volume concentration point is selected after the dead space has been washed out. If the N_2 content of alveolar gas is not uniform, the use of a volume-concentration point early in the alveolar plateau largely eliminates the effects of uneven alveolar N_2 content.   This point is selected by drawing a straight line along the top of the alveolar plateau and extending it to the left.   The point at which the rising N_2 curve first touches this line is taken for measurement of the alveolar concentration.[4]
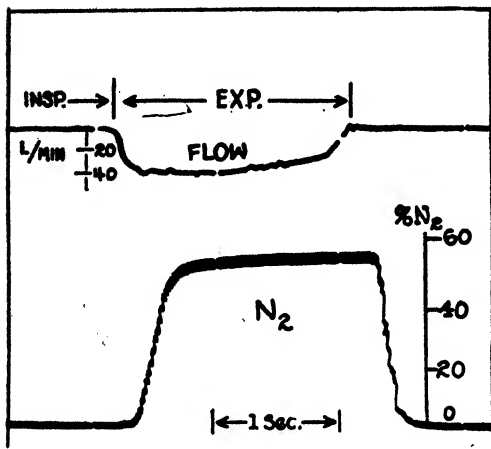
Fig. 1. RECORD OF FLOW and N_2 concentration of expired gas after O_2 inhalation.
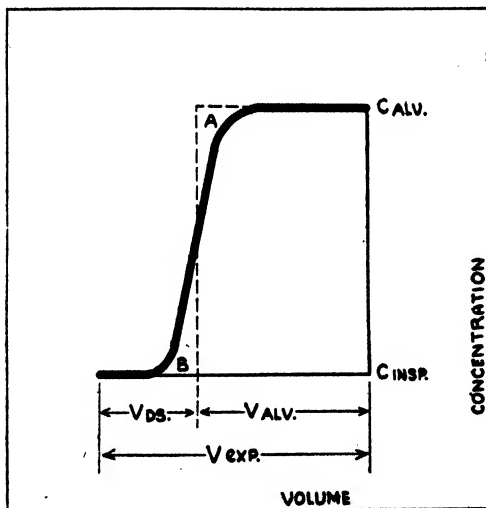
Fig. 2. GEOMETRIC REPRESENTATION of Bohr's formula applied to an expiration (see text for explanation).

Since the preceding calculation is laborious, it was attempted to see how accurately physiological dead space could be estimated directly from the photographic record.   Assuming a constant expiratory flow, an expiration may be represented geometrically by figure 2.   The area under the curve equals $V_e \times C_e$, which by the

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[4] Records of normal subjects show, in practically all cases, an approximately rectilinear plateau; in some subjects with pulmonary disease the plateau is curved and the initial alveolar point cannot be selected in this way.

Bohr equation also equals the sum of the two products, $V_{ds} \times C_i$ and $C_a \times (V_e - V_{ds})$. Since C_i lies on the abscissa, $V_{ds} \times C_i$ contributes no area. Therefore the area under the curve equals $C_a \times (V_e - V_{ds})$. C_a is known, and $V_e - V_{ds}$ may be found by constructing a rectangle which has C_a as one side and which has an area equal to that area under the curve. This is done by dropping a perpendicular to the abscissa such that area A equals area B.

In applying this procedure to the photographic record, a perpendicular is drawn on the nitrogen meter record such that area A equals area B, as in figure 2. This is estimated visually with the help of a squared transparent ruler. The perpendicular is extended to cross the flow curve. The area of the flow curve to the left of the perpendicular is measured, converted to cc. and is called the 'estimated' physiological dead space.

POTENTIAL SOURCES OF ERROR IN METHODS

1. Instrumental Delay. Figure 3 shows an expiratory record (*above*) and a record obtained with a stepwise change in flow and N_2 content (*below*). With quiet
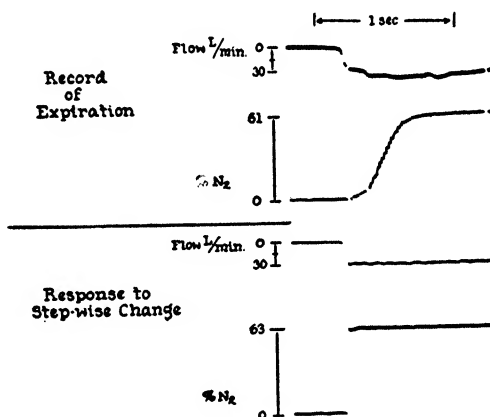
Fig. 3. COMPARISON OF INSTRUMENTAL LAG and changes recorded during expiration (see text for details).

or rapid expiration, the change of N_2 concentration occurs over a period considerably longer (4- to 8-fold and 2- to 4-fold respectively) than the instrumental delay. Likewise the sustained high level of expiratory flow rates is attained only after a delay several times longer than the instrumental lag. Therefore the analyses of N_2 and flow should be reasonable representations of the actual events.

2. The 'estimation' method assumes arithmetic scales on the ordinate and abscissa of figure 2. The non-arithmetic scale of the nitrogen meter response (fig. 1) should make the estimations about 5 per cent too large. Flow variations which occur during the period of rapid N_2 change may introduce error. Actually those flow variations are small because a) more than 75 per cent of peak flow is attained early in expiration, before N_2 beings to rise rapidly, and b) the apparatus has a damping effect. The 'calculation' method is not subject to these errors. Therefore comparison was made between the values obtained by 'calculation' and by 'estimation' in 14 different breaths including quiet and rapid expirations performed by 6 subjects. Estimated values varied both above and below the calculated values. The mean difference between estimated and calculated was 3.4 cc. and the standard error of the mean

difference was ±2.6 cc., indicating that there was no significant difference between values obtained by the two methods.

Further checks on the 'estimation' method were made as follows: *a*) artificial dead spaces were made by connecting rubber hoses to the apparatus mouthpiece, filling the hoses with oxygen and then flushing them through the apparatus with air or gas mixtures containing 63 per cent N_2-37 per cent O_2, or 58 per cent N_2-42 per cent O_2, at flow rates of 15 to 35 l/min. The internal volume of the hoses was measured by filling with water. Three estimations of an 85-cc. smooth bore hose, 2.0-cm. internal diameter, were 89, 93 and 82 cc. Four estimations of a 170-cc. corrugated hose, internal diameter 2.0 to 3.0 cm., were 175, 164, 160 and 175 cc. Six estimations of a 205-cc. corrugated hose were 197, 194, 194, 213, 203 and 206 cc. *b*) Physiological dead space of a subject was 'estimated' to be 168 cc. Then an additional 200 cc. of dead space was added by placing a smooth bore hose in the mouth. Estimations were found to be 373 and 358 cc., differences of 205 and 190 cc.

From the evidence above, it appears that the 'estimation' method, which is technically much easier, is as suitable as the 'calculation'. The data presented here-

TABLE 7. EFFECT OF VARYING INSPIRATORY VOLUME-FLOW ON PHYSIOLOGICAL DEAD SPACE[1]

SUBJECT	PRE-INSP. POSITION	END-INSP. POSITION	INSP. VOL.	INSP. TIME	MEAN INSP. FLOW	PHYSIOL. D.S.
			cc.	*sec.*	*cc./sec.*	*cc.*
34	Max. exp.	Max. exp. + 1760 cc.	1760	1.7	1040	169
	Normal exp.	Max. exp. + 1730 cc.	600	1.5	400	176
30	Max. exp.	Max. exp. + 1570 cc.	1570	1.2	1300	178
	Normal exp.	Max. exp. + 1870 cc.	625	1.2	520	188

[1] All figures average of three breaths.

after were obtained by 'estimation'. In the absence of any direct or absolute method of measurement of physiological dead space (experiments on gas mixing in tubes are not ideal reproductions of aerodynamic conditions in the airway), it is impossible to estimate the accuracy of the values obtained on subjects. These values are, however, similar to those obtained by authors (6) whose methods have eliminated the effects of non-uniformity of alveolar air.

3. Subjective Error in Estimation. In 45 male subjects listed in table 1, estimations of physiological dead space were made by one person on three successive breaths of oxygen, usually the first three breaths. If the physiological and instrumental factors influencing the measurement are assumed to be constant, then the variation between measurements may be said to be due to individual accidental error in the estimation. Although the alveolar N_2 decreases with successive breaths, there was no consistent variation of estimated dead space values between the first and later breaths. The standard deviation of the differences between the individual estimations and the mean of the three was ±8.9 cc. The figures in table 1 represent the mean of the three estimations. In the other experiments where comparisons of change in one individual are made (tables 2-4, 6) single figures represent the mean of

measurements on three successive similar breaths. The standard error of these means is ±5.1 cc., the standard error of the difference between two means is ±7.2 cc. and a difference of 20 cc. or more is significant.

RESULTS

1. The volume of gas required to wash out the dead space on expiration, after which 'alveolar' gas is expired, is shown in table 1 to have an average value of 325 cc. Figure 2 shows that this volume, tentatively called 'kinetic dead space' by Lilly (8), is larger than that of the physiological dead space, since it includes both the pure inspired gas remaining in the airway after inspiration and also some alveolar gas, which is mixed in the airway with inspired gas during expiration.

2. Some alveolar gas appeared in the expired air, as shown by the start of a rising N_2 content, after an average of 41 cc. expired by subjects in table 1. The accuracy of this measurement is limited by the flow meter lag and by the relative insensitivity of this N_2 meter at very low N_2 fractions.

3. Table 1 shows the volume of the physiological dead space found in 45 normal men, sitting and breathing naturally. The average is 156 cc. with an 18 per cent coefficient of variation. Measurements in 4 women gave smaller values, averaging 104 cc.

4. Radiologic and bronchoscopic evidence has shown that the bronchial tree increases in volume with inspiration. Thus, anatomical changes could affect the volume of the physiological dead space. Also the demarcation of gas concentrations between the terminal bronchioles and the alveolar spaces must be affected by diffusion. The effects of these two factors were shown in the following experiments.

A. *Anatomical effects.* Measurements made on 5 men, during quiet breathing and during voluntary hyperventilation, are shown in table 2. Large increases in tidal volume resulted in increases of 46 to 95 cc. in physiological dead space. A comparison of voluntary hyperventilation and hyperpnea due to exercise (one minute after knee bends) was made in 2 subjects. Table 3 shows that there was no significant difference between the increased physiological dead space found with the two types of hyperpnea. In view of Verzar's data on changes in total lung volume during and after exercise (20), it is possible that different results would have been obtained on measurements during exercise, which were precluded by apparatus immobility. The significance of the notation of inspiratory time in table 3 will be discussed below.

The increased physiological dead space found with larger tidal volumes is presumably due to the increase in anatomical volume on deeper inspiration. Further demonstration was obtained by experiments on 2 subjects in whom oxygen inhalation was started at *a)* maximal expiratory position, *b)* normal quiet expiratory position and *c)* almost maximal inspiratory position. The maximal differences in end-inspiratory lung volumes were 3000 to 3500 cc.; tidal volume and rate were voluntarily made similar at all three lung positions. Table 4 shows that at reduced lung volumes the physiological dead space was definitely decreased. With an increase of about 3000 cc. in lung volume, the physiological dead space increased about 100 cc.

B. *Diffusion effects.* Experiments were made on 5 subjects in which the time available for diffusion of N_2 between terminal bronchioles and the more peripheral spaces was prolonged by holding the breath for about 20 seconds in the normal inspiratory position. Table 5 shows a definite decrease in physiological dead space with breathholding in every case, presumably because the peripheral boundary of pure inspired gas had receded up the bronchial tree.

In 3 male subjects, diffusion time was varied within physiological limits by voluntarily breathing with *a)* rapid inspiration and expiration and *b)* slow inspiration and expiration. In 2 subjects,

breaths with a short end-inspiratory pause were alternated with uninterrupted breaths. Table 6 shows that the physiological dead spaces were smaller with the slow rate and that 2- to 3-second inspiratory pauses also resulted in a decrease.

5. To test the proposal (5) that physiological dead space is decreased by higher rates of inspiratory flow, experiments were done on 2 subjects in which inspiratory time and end-inspiratory lung volume were constant, but the rate of inspiratory volume flow was varied. This was accomplished by analyzing breaths which ended at similar inspiratory positions but started at varying expiratory levels. Inspired volumes were measured on a 6 L. recording spirometer used for the source of O_2. Table 7 shows that increasing the mean inspiratory volume flow by about 2.5 times did not significantly affect the volume of physiological dead space.

<div align="center">DISCUSSION</div>

Since the physiological dead space may change in any one individual with respiratory rate and depth, a 'normal' value for a group with varying rates and depths of respiration, and varying anatomy, is not very meaningful. However the average value of 156 cc. for resting males agrees closely with the commonly accepted value of 150 cc. and the 104–cc. average for 4 females is similar to Lindhard's average value of 92 cc. for 5 females (10). On the other hand, Kaltrieder *et al.* (11) found an average value of 256 cc. in a group of 50 males, 38 to 63 years old; if the non-uniformity of alveolar ventilation is increased in older 'normal' males (1), a larger value would be expected when calculations are based on Haldane-Priestly alveolar air samples. The method described in this paper has the advantages of knowing the concentration of alveolar gas which immediately follows that alveolar gas which washes out the dead space and of not requiring the cooperation of the subject in obtaining a sample of alveolar gas. It is probable that these factors are partly responsible for the smaller coefficient of variation in this group (18%) as compared to that in Kaltrieder's series (43.5%).

A recent text (12) illustrates the calculation of alveolar ventilation with *a*) a dead space of constant volume and *b*) a constant dead space/tidal volume fraction. Our data show that dead space volume is not constant; also it is a variable fraction both of tidal volume in different individuals and of different tidal volumes in one person.

Many authors have found that physiological dead space increases with increasing lung volume. However, in our experiments, the increase from maximal expiratory position to maximal inspiratory position was only of the order of 100 to 150 cc., as Krogh (3) and others (6, 13) have also found. Combining the anatomical data of Rohrer (14) and the radiological data of Huizinga (15), one can calculate that the volume of the airway, from the glottis to the intralobular bronchioles of the fifth order, would increase by about 230 cc. from deep expiration to deep inspiration. The smaller increase measured by physiological methods may perhaps be explained by the time that elapses between the first exposure, during early inspiration of inspired gas in the bronchioles, to the gas of alveolar ducts and alveoli and the exit and analysis of this gas on the succeeding expiration. During this time, diffusion exchange will occur between the bronchioles and the alveoli and the dead space measured by physiological methods will be smaller than the anatomical volume.

Henderson *et al.* (16) and Haldane (17) found that the dead space for CO_2 and O_2 decreased if the breath was held after inspiration; Grosse Brockhoff and Schoedel (5) were unable to confirm this. Krogh and Lindhard (3) and Mundt (6), using hydrogen methods, noted a decrease but it is doubtful if results obtained with such a rapidly diffusing gas should be applied to the normal respiratory gases. Our data show that prolongation of inspiratory time by only two seconds, such as may result from a prolonged inspiration, will decrease significantly the physiological dead space. Thus with deep slow respiration, the anatomical increase will be counteracted by the prolonged time available for diffusion between the terminal bronchioles and the alveoli. The finding that a 20-second prolongation of inspiratory time (table 5) does not reduce the physiological dead space a great deal more than a two- to four-second prolongation (table 6) is not surprising. In the respiratory bronchioles, for example, the diffusion course is short and only a few seconds or less are required to greatly reduce initial concentration differences (19). The effect of diffusion in the larger bronchioles will be less evident because their total volume becomes progressively smaller toward the large bronchi; also as diffusion courses become longer, time must be prolonged exponentially to accomplish similar degrees of concentration equalization.

Our finding that physiological dead space is unaffected by increased inspiratory volume flow does not support Grosse-Brockhoff and Schoedel's proposal that bronchiolar-alveolar mixing is facilitated by turbulence resulting from faster inspiratory flow, with a resulting reduction of dead space volume. While it cannot be said that turbulent flow and attendant mixing do not occur, diffusion alone should accomplish mixing between the main stream and the alveoli along the respiratory bronchioles and alveolar ducts because the distances involved are so small in relation to normal respiratory times.

The measurements of respiratory dead space by the method described may have several clinical uses: *a*) patients with pulmonary abnormalities may have large ineffective tidal volumes (2, 18); this method may be able to demonstrate whether an enlarged physiological dead space or uneven intrapulmonary gas mixing is responsible. *b*) It may be possible to employ the method to measure physiological or pharmacologically induced changes in bronchiolar caliber.

SUMMARY

1. Physiological dead space was measured by simultaneous and continuous measurement of volume flow and N_2 content of gas expired following the change from breathing air to breathing 99.6 per cent O_2. In normal subjects the effect of non-uniform alveolar gas on dead space measurements can be largely eliminated.

2. The average volume of the physiological dead space in 45 healthy males at rest was 156 cc.; the average expired volume required to wash out the dead space was 325 cc. The physiological dead space/tidal volume fraction averaged 25.9 per cent, but varied widely in different individuals.

3. The volume of the physiological dead space is affected by: *a*) anatomical volume of the bronchial tree. Maximal variations of inspiratory lung volume changed dead space by about 100 cc. Voluntary hyperventilation and post-exercise hyperpnea increased physiological dead space equally; the increase was 100 cc. or

less. *b*) Gas diffusion between terminal bronchioles and alveolar spaces. Prolongation of inspiratory time by two to three seconds significantly reduced the volume of the physiological dead space and breathholding, during inspiration (20 sec.), caused reductions of 44 to 82 cc. In slow deep breathing the anatomical volume increase is counteracted by diffusion occurring during the prolonged inspiratory time.

4. Variations in the rate of inspiratory volume flow did not affect the volume of the physiological dead space.

5. Clinical applications of the method are suggested.

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RÔLE OF THE VAGI IN THE CROSSED PHRENIC PHENOMENON

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THE term 'crossed phrenic phenomenon' refers to the recovery of activity in a hemidiaphragm paralyzed by hemisection of the spinal cord above C3, when the contralateral phrenic nerve is cut or blocked in various ways.

This phenomenon was observed by several earlier workers (1–3) and it was found (3) that the crossing of descending respiratory impulses occurred at the level of the phrenic nuclei.

Deason and Robb (4) showed that crossing could be produced (in dogs and cats) under several conditions, even without previous phrenic section. Thus they found crossing to occur with 'dyspnoea' (not further described), traction on the active phrenic not great enough to stop conduction and on stimulation of the sciatic.

Rosenblueth and Ortiz (5) found that crossing occurred—on section or block of the active phrenic—in dogs, cats, rabbits and woodchucks, but not in monkeys or guinea pigs. In dogs only, crossing also occurred regularly on section of the vago-sympathetic trunks. They concluded that the crossing was not due to asphyxia or to interruption of inhibitory afferent impulses in the phrenic or vago-sympathetic nerves.

After a subsequent study Rosenblueth, Klopp and Simeone (6) were forced to conclude that crossing is due to block of phrenic motor impulses and that the central changes—at the level of the phrenic nuclei—which permit crossing are mediated by some process not involving the conduction of nerve impulses.

Tosatti (7) found that the phrenic nucleus has a double innervation from the respiratory center—a principal homolateral tract running in the lateral column of the cervical cord and a reserve tract running in the anterior column and composed of both direct and crossed fibers. He held that crossing was due to activation of this crossed pathway, perhaps by elevation of the blood CO_2 concentration, which he showed occurred after inactivation of the functioning phrenic.

Finally Seligman and Davis (8) found that crossing was produced by prostigmin, eserine, acetylcholine (protected by prostigmin) and strychnine. They also showed that crossing occurred with asphyxia and after section of the vagi if prostigmin had been given previously.

Thus in several species crossing can occur, under various conditions, without interruption of the remaining phrenic motor activity. The present study is a further investigation of the crossed phrenic phenomenon with emphasis on the rôle of the vagi.

METHOD

Our conclusions are based on observations made on 23 rabbits. At first dial (Ciba, o.5 cc/kg.) and later nembutal (Abbott, o.4–o.6 cc/kg.) were used as anesthetics intravenously. The kind of anesthetic used did not appear to affect the results, although the rabbits tolerated nembutal better than dial.

Diaphragmatic contractions were at first recorded from Head's slips (anteriorly

placed bands of diaphragmatic fibers, well developed in the rabbit) with isotonic levers—in such records inspiratory excursions are upward. Later records were made directly from the domes of the diaphragm after the abdomen had been widely opened. In these records the inspiratory excursions are downward. The use of Head's slips was abandoned when it become apparent that in many animals the slips did not contract even when the diaphragm was contracting well. We came to regard Head's slips as accessory muscles of respiration, since contraction could often be induced in quiescent slips by having the animal breathe against resistance.

The respiratory effects of all procedures used were checked by direct visual observation of the diaphragm.

In all animals a tracheal cannula was inserted and the cord hemisected between C_2 and C_3 to produce a respiratory hemiplegia. The vagi and phrenics were approached in the neck. The phrenics were inactivated by section or by block with ether or ice. Stimulation was produced through shielded electrodes using a Grass model 3 monophasic square-wave stimulator with variable pulse duration, frequency and intensity.

<div align="center">RESULTS</div>

A. *Rôle of Hering-Breuer Afferents.* As has been mentioned, in some animals with a cervical hemisection Head's slips did not contract with respiration even on the unparalyzed side. In such circumstances, partial occlusion of the tracheal cannula led to contraction of the slips both on the unparalyzed and occasionally on the hemiplegic side. As can be seen in figure 1, the contractions started with the very next breath after partial occlusion, a characteristic of Hering-Breuer afferent activity.

Even when the normal Head's slip was contracting, partial occlusion of the tracheal cannula increased activity on the functioning side and sometimes induced activity on the hemiplegic side.

The crossing produced by partial occlusion could also be shown on records from the domes of the diaphragm (fig. 2A). That this increase in activity was due in part at least to impulses in the vagi was shown by the different character of the response to occlusion after the vagi had been sectioned bilaterally (fig. 2B). In these circumstances respiration did not slow or increase as greatly as when the vagi were intact. Possibly the increased depth and crossing of respiration on occlusion· after vagal section was due mostly to asphyxia, since with vagi cut the increase was more gradual, did not become maximal until the animal was visibly cyanotic and persisted longer after the airway had been reopened.

Crossing produced by partial occlusion of the tracheal cannula may be related to the crossing described by Deason and Robb (4) which occurred when their animals were in a state of 'dyspnoea'.

B. *Effects of Section of the Vagi.* As noted above, Rosenblueth and Ortiz (5) found that crossing was readily produced by severing the vago-sympathetic trunks in dogs, but not in monkeys, cats, rabbits, guinea pigs or woodchucks. Rosenblueth, Klopp and Simeone (6) showed that sectioning the vagi in cats and rabbits would produce transient crossing if crossing had been produced previously by one or more reversible blocks of the active phrenic. Seligman and Davis (8) produced permanent

crossing on vagal section in one cat and in rabbits which had received prostigmin previously.

In the present experiments, section of the vagi produced transient crossing in 4 out of 8 animals in which it was performed as the initial experimental procedure (fig. 3). The rôle which the vagi may play in the production of crossed respiration is indicated by the fact that in the 4 animals which did not show crossing on vagal section, no crossing occurred on subsequent phrenic block. In addition there was a correlation between this lack of crossing and an inactivity of Hering-Breuer reflexes as indicated by the responses to partial occlusion of the tracheal cannula and to compression of the thorax from without. Normally partial occlusion slowed respiration while compression of the thorax accelerated it. But in a typical animal from
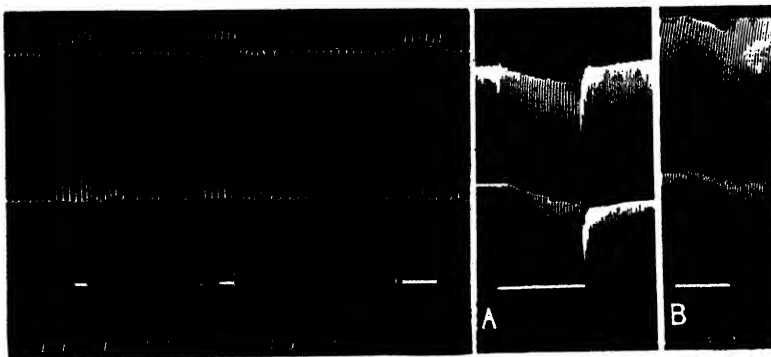
Fig. 1 (*left*). RECORD MADE FROM HEAD'S SLIPS. The upper record is from the normal side; the lower, from the hemiplegic side. This and all subsequent records are from rabbits given a respiratory hemiplegia by cervical hemisection above C3. In this case, Head's slips were not functioning. At signals, partial occlusion of the tracheal cannula. Note that this procedure activated both the normal and hemiplegic slips. Activation occurred with the very next breath after partial occlusion of the cannula. Time interval (bottom signal) in this and all subsequent figures is 10 seconds.

Fig. 2 (*right*). RECORDS FROM THE DOMES OF THE DIAPHRAGM. Upper record, normal side; lower record, from hemiplegic hemidiaphragm. *A*. Vagi were intact. At signal, partial occlusion of the tracheal cannula caused crossing which persisted somewhat when the airway was reopened. *B*. Same preparation as in *A*, but vagi have been cut, producing slight crossed activity in the previously hemiplegic hemidiaphragm. At signal, partial occlusion of the tracheal cannula. Note difference in the response of the hemidiaphragms from that of fig. 2A. The respiratory movements did not slow or increase as much as they did with vagi intact.

the 'non-crossing' group mentioned above, Hering-Breuer responses were poor, section of the vagi did not produce crossing and ether block of the phrenic was ineffective in producing crossing both before and after bilateral vagotomy.

C. *Effects of Stimulation of Central End of Cut Vagus.* Since partial occlusion of the tracheal cannula could cause crossing, an effect some of which depended on the integrity of the vagi (see above), an attempt was made to reproduce this phenomenon by appropriate stimulation of the central end of the cut vagus. It is now well known (9) that high frequency vagal stimulation results in a slowing of respiration and an expiratory apnea, while low frequency stimulation causes a quickening of respiration and an inspiratory apnea. Accordingly the central end of the cut vagus was stimulated with monophasic square waves of 0.01 msec. duration at rates of 120 to 300 per second. Such stimulation caused the appearance of, or an increase in

crossed activity, an effect which might persist after stimulation had ceased (fig. 4A). That this increase in crossing was probably not due to anoxia from the apnea initially produced by high frequency stimulation is demonstrated in figure 4B, where an increase in crossed activity is shown even though the animal had been breathing 100 per cent O_2 for several minutes before stimulation of the vagus.

Since crossing may occur on section of the vagi, the possibility of inhibitory afferents in the vagi was investigated. Appropriate low-frequency (5 per sec.) stimulation with square waves as before was found to inhibit crossed as well as normal respiratory diaphragmatic movements.

We conclude then that the vagus contains afferent fibers which both stimulate and inhibit crossed respiration. The appearance of crossing after vagal section in some animals could then be due to removal of this inhibition. It might also be
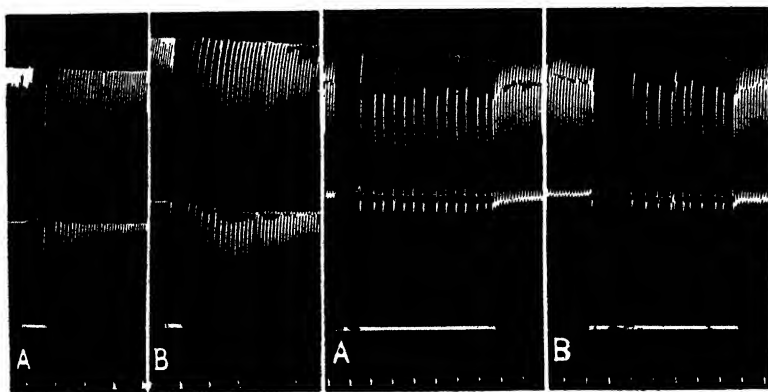
Fig. 3 (*left*). EFFECTS OF SECTIONING VAGI. Upper record from normal hemidiaphragm; lower record from hemiplegic hemidiaphragm. Records from domes. *A*. At signal, ligation and section of one vagus. Crossing produced. *B*. Same preparation. At signal, subsequent section of the other vagus. Crossed diaphragmatic contractions again increased.

Fig. 4 (*right*). SHOWING CROSSING PRODUCED BY HIGH FREQUENCY VAGAL STIMULATION (at signal). Lower record is from the hemiplegic hemidiaphragm; upper record from normal side. Records from domes. *A*. With animal breathing air. Stimulation produced a short period of apnea and increased crossed contractions. *B*. Same preparation, after animal had been breathing 100 per cent O_2 for several minutes before stimulation. Vagal stimulation still caused crossing.

inferred that crossing after inactivation of the functioning phrenic could likewise be caused by a decrease in vagal inhibition, or by an enhancement of the stimulatory impulses, brought about by some peripheral change.

D. *Relation of the Vagi to Crossing Produced by Block of Active Phrenic.* In spite of the rôle which the vagi may play in the appearance of or inhibition of crossed respiration, block of the active phrenic after crossing had been produced by bilateral vagal section still caused an increase in crossed activity (fig. 5). Asphyxia may play some part in this increased crossed respiratory activity after block of the active phrenic, but it does not furnish the whole explanation. An animal with both vagi cut still showed increased crossing on phrenic block even after it had been breathing 100 per cent O_2 for several minutes previously. Interestingly enough, under these circumstances the crossed activity did not develop to the extent it would have had the animal been breathing air. This result indicates that whatever the mechanism

which brings about the onset of crossed respiration, the crossed pathway—once active—responds as does the normal respiratory pathway to physiological stimuli.[2]

E. *Possible Rôle of Inhibitory Afferents in the Phrenic.* Deason and Robb (4) found that stimulation of the central end of the cut phrenic in cats and dogs increased the rate and amplitude of respiratory movements. They felt that crossing might be initiated by excitatory afferent discharges from the cut end of the nerve.

Rosenblueth and Ortiz (5) stimulated the central end of the cut active phrenic with an inductorium in dogs, cats, rabbits and woodchucks, obtaining variable results. During the present investigation the central end of the cut active phrenic was stimulated with monophasic square-waves whose duration varied from 0.01 to 20 msec., with intensities as high as 100 volts, and with frequencies ranging from 1 to 500 per second. In no instance was any significant effect on respiration noted, though the existance of afferent fibers in the phrenic affecting respiration has been postulated (10–13). On the basis of the available evidence we agree with Rosenblueth and Ortiz that crossing does not result from the removal of inhibitory impulses in the active phrenic.
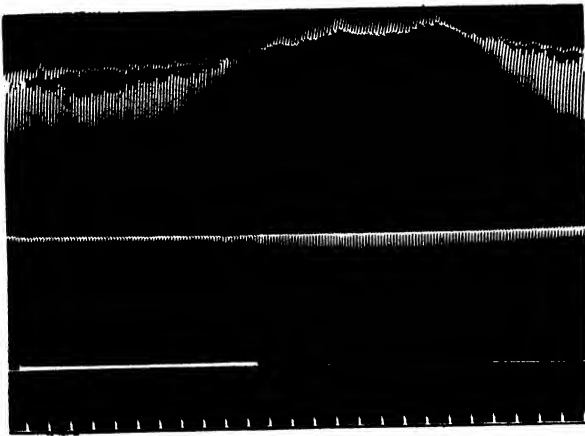
Fig. 5. RECORDS FROM DOMES. Upper, normal side; lower, from hemiplegic hemidiaphragm. Slight crossed activity had resulted from section of both vagi. At signal, ether block of active phrenic. Note further increase in crossed activity after phrenic block.

DISCUSSION

It seems obvious that crossing of descending respiratory impulses may occur under various conditions and be brought about by several factors that affect respiration—e.g. asphyxia, drugs and afferent stimulation. From our results it would be tempting to formulate an hypothesis for the crossed phrenic phenomenon based on changes in afferent impulses in the vagi and other nerves brought about by section or block of the active phrenic, with a resulting stimulation of or removal of inhibition from the crossed respiratory pathway. Such an hypothesis could even account for the appearance of, or increase in, crossed respiration on phrenic block after previous section of the vagi, since it has been shown that the lung is supplied with afferents other than those running in the vagi. These afferents have been demonstrated in various species by several investigators (14–17).

Such an hypothesis may account for crossing under certain conditions. How-

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[2] We have also been able to activate the crossed pathway directly with the respiratory stimulant nikethamide (Coramine, kindly furnished by Ciba Pharmaceutical Products, Inc., Summit, N. J.). (Unpublished data.)

ever, Rosenblueth, Klopp and Simeone (6) showed conclusively that crossing can occur independent of any change in afferent activity and, under these circumstances, must depend essentially on some change in the phrenic nuclei brought about by severance of the phrenic nerve. The nature of this change remains the most interesting aspect of the crossed phrenic phenomenon. The recent claim of Dolivo and Fleisch (18) that sectioning one phrenic increases the electrical activity of the opposite phrenic, even when all afferent nerves affecting respiration have been sectioned, seems pertinent to a solution of this problem.

SUMMARY

The 'crossed phrenic phenomenon' consists of the reactivation of a hemiplegic hemidiaphragm by section or block of the contralateral active phrenic. Experiments on rabbits are reported with reference to the rôle of the vagi in this phenomenon. Crossed respiration was produced by activation of Hering-Breuer afferents by partial occlusion of a tracheal cannula (section A, figs. 1 and 2). Crossing was produced by section of the vagi alone (section B, fig. 3).

Four animals which did not cross on phrenic block or on vagal section showed poor respiratory responses to partial tracheal occlusion and external compression of the thorax (section B). High frequency stimulation of the central end of the cut vagus stimulated crossing (section C, fig. 4). Low frequency stimulation of the vagus inhibited crossing (section C). After vagal section had caused crossing, an increase in crossing still occurred after inactivation of the functioning phrenic (section D, fig. 5). No afferents inhibitory to respiration could be demonstrated in the phrenic nerve with the technic used (section E). The discussion is concerned with the hypothesis that under certain conditions crossing could be produced by changes in afferent impulses from the lungs resulting from inactivation of the phrenic. It is emphasized that the essential problem of the crossed phrenic phenomenon remains to be solved.

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BLOOD SUGAR AND DEXTROSE TOLERANCE DURING ANOXIA IN THE DOG[1]

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RELATIVELY few studies of the blood sugar response of dogs to anoxic anoxia have been reported. McQuarrie *et al.* (1) have determined the hyperglycemic response of fasting dogs exposed for 2.5 hours to 4.5–5.0 per cent O_2, an extreme degree of anoxia, after a gradual transition from room air. They found increases of between 30 and 150 per cent in the blood glucose level. Lewis *et al.* (2) found no change in blood glucose levels at the end of 24 hours at a pO_2 of 80 mm. Hg. As Kelley and McDonald (3) have recently stated, there are contradictory reports in the literature regarding the effects of anoxia on dextrose tolerance. They found that it was decreased in their 3 dogs at simulated altitudes of 18,000 and 24,000 feet.

We have determined, in strictly unacclimatized dogs, the threshold degree of anoxia which will produce hyperglycemia, the extent of the hyperglycemia at various altitudes and the change in blood glucose level over time at a simulated altitude of 28,000 feet. We have also determined the dextrose tolerance in 7 dogs at a simulated altitude of 28,000 feet.

METHODS

Ninety healthy normal dogs which, to our knowledge, had never before been exposed to anoxia and which had been fasted approximately 20 hours, were used to study the hyperglycemic response to anoxia. These dogs were exposed without anesthesia to the five simulated altitudes (in a decompression chamber) and for the time intervals shown in table 1. The fasting blood sample was drawn from the saphenous vein immediately before the ascent to the simulated altitude. (Blood samples taken in 20 dogs immediately before and after a 15-minute stay in the decompression chamber at ground level showed that the blood sampling procedure did not elevate the blood sugar in the average dog.) The ascent to the simulated altitude was made rapidly, that is, in about 70 to 160 seconds, depending upon the altitude. The descent at the end of the period of anoxia took 20 to 25 seconds. The second blood sample was drawn within 1.25 minutes on the average after descent.

In 42 of the dogs hemoglobin was determined on the fasting blood sample by the Sahli method.

Seven dogs were used for the studies on dextrose tolerance, but exposure to altitude or the administration of glucose were not repeated more often than once a week in order to minimize the cumulative effects. After taking the fasting blood sample, 1.5 gm. of glucose per kg. of body weight were injected as a 25 per cent solution intravenously. The dog was then exposed immediately to a simulated altitude of 28,000 feet. At 15, 30, 60 and 90 minutes after the beginning of the ascent the dog was returned briefly to ground level (2 min. on the average) for blood sampling. For the dextrose tolerance determination at ground level, the blood samples were drawn at corresponding time intervals. The hyperglycemic response of these dogs to 28,000 feet was determined on blood samples drawn similarly. In all but one dog each procedure was repeated three times.

All blood sugar determinations were made by the method of Folin and Wu.

RESULTS

The blood sugar responses for various intervals of time to various degrees of anoxia are given in table 1. It will be seen that 24,000 feet was the lowest simulated altitude at which a statistically significant elevation of blood sugar occurred when the duration of exposure was 15 minutes. The same duration at 28,000 feet produced a much greater elevation and at 32,000 feet the response was still more marked. The average blood sugar level after 15 minutes at the latter was significantly greater than at 28,000 feet (p: 0.007).

The hyperglycemic response at 28,000 feet was greatest after an exposure of 30 minutes. As the length of exposure was increased the hyperglycemia at the end of the time interval became less till after 60 minutes the blood sugar level was below that after 15 minutes. It should be emphasized that in order to avoid the use of dogs adapted to anoxia in any degree, the response for each altitude and/or duration of exposure was determined on dogs which had never been used before.

TABLE 1. BLOOD SUGAR RESPONSE OF FASTED DOGS EXPOSED FOR VARIOUS INTERVALS OF TIME TO VARIOUS DEGREES OF ANOXIA

BAROMETRIC PRESS.	SIMULATED ALT.	LENGTH OF EXPOS.	NO. OF DOGS	FASTING BL. SUGAR	FINAL BL. SUGAR	DIFF.	'P'	PER CENT DIFF.
mm. Hg	*ft.*	*min.*		*mg/100 ml.*	*mg/100 ml.*	*mg/100 ml.*		
382	18,000	15	10	111	116	5	>0.20	4.5
382	18,000	30	6	102	104	2	>0.20	2.0
328	22,000	15	10	104	108	4	>0.20	3.8
303	24,000	15	10	115	121	6	0.009	5.2
254	28,000	15	14	104	145	41	<0.001	39.4
254	28,000	30	10	110	176	66		60.0
254	28,000	45	7	113	164	51		45.1
254	28,000	60	7	109	131	22		20.2
208	32,000	15	16	110	166	56		50.9

The extent of the elevation of blood sugar produced by anoxia was negatively correlated with the fasting blood hemoglobin concentration, but the degree of correlation was quite low (r: −0.42; N: 42).

The dextrose tolerance curves at ground level and at 28,000 feet along with the hyperglycemic response of the same dogs are shown in figure 1. The curves are the average results on 7 dogs and are expressed as per cent increases above the fasting blood sugar level which averaged 109 mg/100 ml.

The dextrose tolerance curve during anoxia had a maximum at 15 minutes which was somewhat below that at ground level. In spite of this, at 60 and 90 minutes the curve was elevated and tended to approach the curve of the simple hyperglycemic response to anoxia as the control dextrose tolerance curve approached the fasting level. The average blood sugar levels at 60 and 90 minutes of the dextrose tolerance curve during anoxia were significantly (p: 0.01) elevated above the corresponding ones without anoxia. This, of course, indicates that dextrose tolerance was decreased.

A comparison of the hyperglycemic response to simple anoxia (lower curve of fig. 1) when the exposure was interrupted by descents to ground level with that when it was not so interrupted (table 1) reveals that the two were not alike in all aspects. The maximum occurred in the former at the end of 15 minutes while in the latter it occurred at the end of 30 minutes where it was significantly greater (p: 0.008). The blood sugar levels at 15 and 60 minutes, however, were about the same for both.
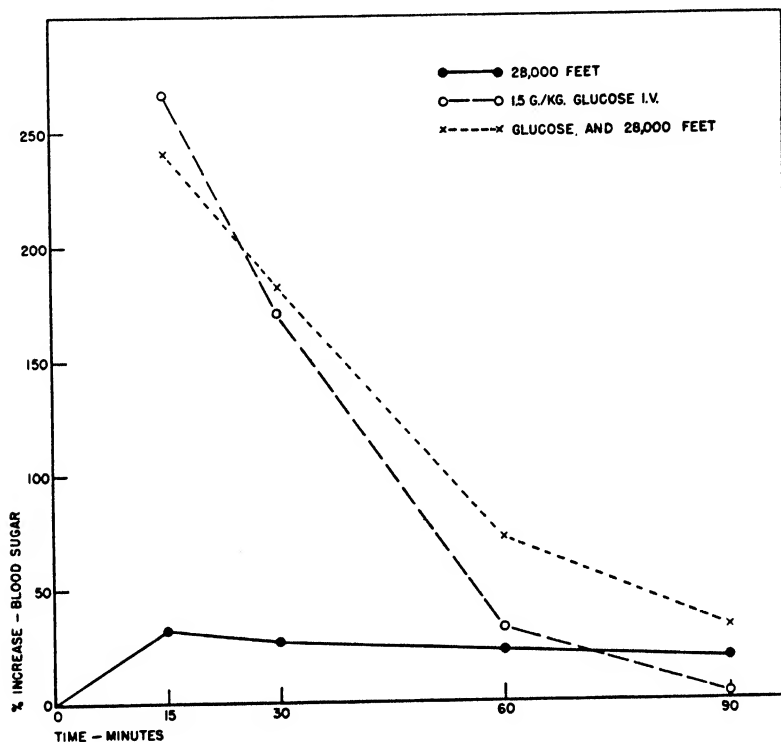
Fig. 1. EFFECT OF ANOXIA on the dextrose tolerance of 7 dogs. Dextrose tolerance at ground level (open circles); dextrose tolerance at 28,000 feet (crosses); hyperglycemic response to interrupted anoxia (filled circles).

DISCUSSION

McQuarrie *et al.* (1) have shown that in the dog the adrenal glands are necessary for the hyperglycemic response to anoxia. The blood sugar level reached, however, is due to the participation of both the sympathetic (adrenals) and parasympathetic (pancreatic islets) divisions of the autonomic nervous system in which the former is dominant. The secretion of epinephrine, apparently, is responsible for the blood sugar elevation during relatively short exposures to anoxia.

The work reported here indicates that an altitude of 24,000 feet for 15 minutes can produce a threshold mobilization of blood sugar. Higher altitudes for a similar length of time cause a proportionate increase in the response. The failure of Kelley and McDonald (3) to find an elevation of blood sugar at 24,000 feet can be ascribed

to the short length of exposure (approximately 5 min.), possibly to their failure to use strictly unacclimatized dogs and to the limited number of experimental animals used. The rôle of the sympathico-adrenal system in elevating blood sugar under greater anoxic stress can not be called into question by the results reported by the above authors. The threshold in anoxic stress which will initiate other adaptive reactions of the body is considerably below that for hyperglycemia. The mobilization of blood sugar has long been recognized as an emergency response of the homeostatic mechanisms (4). As might be expected, the duration of exposure is a factor in setting in motion such mechanisms. Thus, the results of our study indicate that a 30-minute uninterrupted exposure to 28,000 feet is as effective as a 15-minute one to 32,000 feet in elevating blood sugar in dogs. Continued exposure at 28,000 feet results in a decline in blood sugar level. Gellhorn and Packer (5), in rabbits, have concluded that prolonged anoxia leads to a loss of the earlier glycogenolytic activity of epinephrine, the secretion of which was presumed to continue. However, the possibility that epinephrine secretion may decline under such circumstances or that other hormonal and/or nervous mechanisms may intervene, have not been strictly excluded.

The percentage increase in blood sugar at the end of 15 minutes in the dextrose tolerance test at altitude was not quite so great as at ground level. The additional mobilization of blood sugar by anoxia might have been expected to cause this point to be elevated above that of the control. This mobilization may not have been present, for Safford and Gellhorn (6) have shown that in the rat the reactivity of the sympathico-adrenal system decreases with rising blood sugar level. It is not apparent (inasmuch as no obvious correlation between fasting blood sugar levels and blood sugar rises due to anoxia could be found in our results) whether or not such an effect holds for the dog. Another possibility is the greater urinary loss of glucose in anoxia. From the work of Toth (7) on unanesthetized dogs it could be predicted that urine excretion would be elevated some 50 to 65 per cent by the degree of anoxia in question here. Since the blood sugar level was above the renal threshold in our experiments, one would have expected a greater loss through glycosuria in the dogs at altitude than at ground level.

The elevated points in the dextrose tolerance curve during anoxia at 60 and 90 minutes are evidence that tolerance was decreased. From inspection of the records, the decreased tolerance appears to be due to the persistence of the hyperglycemic response to anoxia. If the sympathico-adrenal system had initially been suppressed (as suggested by Safford and Gellhorn) by the artificially produced high blood sugar level, it seems to have been activated as the experiment progressed. It is possible that other factors may be involved as suggested by Kelley and McDonald (3): *a*) insufficient oxygen for enzymatic systems involving carbohydrate; *b*) increased adrenal cortical activity. Our results, although obtained under conditions differing in important aspects, confirm those of the latter authors in respect to dextrose tolerance during anoxia in the dog.

SUMMARY

The subjection of 90 strictly unacclimatized dogs to simulated altitudes in a decompression chamber has shown that the threshold degree of anoxia for invoking

hyperglycemia in the average dog is that at 24,000 feet for 15 minutes. Exposures to 28,000 and 32,000 feet for the same length of time invoke proportionately greater elevations of blood sugar. When length of exposure at 28,000 feet was varied from 15 to 60 minutes the maximum rise was seen at the end of 30 minutes. Blood sugar level then declined with continued exposure.

Dextrose tolerance at a simulated altitude of 28,000 feet was found to be significantly decreased on the average in the 7 dogs studied.

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RÔLE OF THE HERRING-BREUR REFLEX UNDER DEEP PENTOTHAL ANESTHESIA

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SEVERAL reports have appeared in the literature on the stimulating effect to respiration of pressure on the thoracic wall (1, 2). More recently reports have been published on a reflex response purportedly originating in the thoracic wall of the dog during periods of respiratory arrest induced by intravenous pentothal (3, 4). According to these observers the respiratory response was elicited by light pressure applied to the region of the fourth rib, at a point of common insertion for the scalenus medius and rectus abdominis muscles in the dog. The assumed reflex basis for these results has been questioned (5, 6). The observations to be reported here were undertaken with the hope of quantitatively evaluating the strength of stimulus necessary to elicit this response. Later the experimental procedure was applied to a study of the effects of unilateral and bilateral vagotomy and of unilateral pneumonectomy.

PROCEDURE

Fifty-six experiments were performed on 40 some dogs. The animals were anesthetized by intraperitoneal injection of 35 mg. nembutal or pentothal sodium per kg. Oxygen was given by intratracheal insufflation at a rate of 0.5 to 3 liters per minute. Intrapulmonic pressure was maintained at atmospheric values. Cyanosis was absent. Respiration was recorded by a tambour connected to an endotracheal tube. The experimental procedure consisted in studying the response to stimulation during periods of respiratory arrest induced by intravenous pentothal or nembutal. In the earlier experiments attempts were made to maintain a constant depth of respiratory depression by controlling the rate of intravenous drip of 2.5 per cent pentothal. In later experiments 1 cc. or 2 cc. of 2.5 per cent pentothal was injected intravenously and periods of arrest varying from a few minutes to over an hour were obtained. In some cases similar results were obtained by using 2.5 per cent nembutal intravenously.

In order to evaluate the effectve strength of stimulus, a 1000-gm., 500-gm. or 200-gm. weight was applied to a freely moving piston in contact with the chest wall. The approximate rate of recovery of excitability was estimated by determining the minimal stimulus at 10-second intervals during the period of inhibition. Similar observations were made on 9 dogs subjected to unilateral or bilateral vagotomy and on 6 dogs with unilateral pneumonectomy. In 4 of the latter, care was taken to avoid injury to the scalenus and rectus muscles during the operation. Respiratory rates were also determined on some of these animals under normal unanesthetized conditions for periods up to eight months following the operation.

RESULTS

Quantitative measurements of the minimal stimulus during periods of arrest were only partially successful. The duration and depth of inhibition varied with the dose

and the interval of time between injections. If 1-cc. doses of pentothal were used and a five-minute period of spontaneous breathing between periods of inhibition was allowed, fairly reproducible curves could be obtained. No attempt was made to estimate the concentration in the blood or rate of elimination of the anesthetic. Figure 1 is shown to indicate the type of result commonly obtained with 1-cc. doses of 2.5 per cent pentothal. Nembutal produced longer and apparently shallower periods of inhibition. Reflex response to the weight could be elicited readily in a zone extending from the second to the sixth rib along the sternal margin. Areas of the thoracic wall bordering the spinal column were less sensitive. Section of the scalenus medius and rectus abdominis muscles did not abolish the reflex response during pentothal inhibition. On the other hand, stretching these muscles failed to elicit a response. In two experiments the reflex was obtained by applying light finger pressure to the parietal pleura after resection of the fourth rib and removal of the adjacent thoracic wall. Responses were mainly diaphragmatic and in some instances
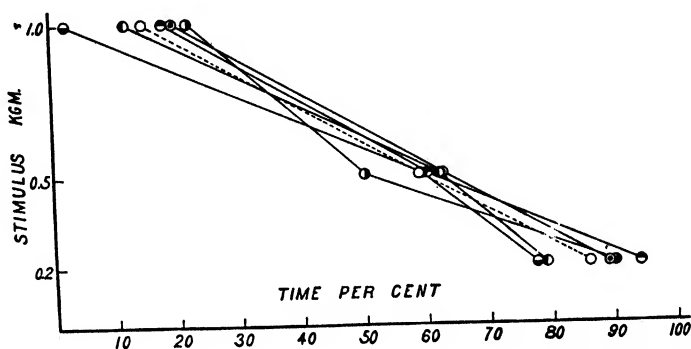
Fig. 1. MINIMAL STIMULUS DURING PERIODS OF PENTOTHAL INHI-BITION. The broken line represents the average of a series of runs on the same dog. Due to the variability in duration of inhibition (280 sec. to 580 sec.) the percentage of total time is used.

a subminimal stimulus was observed to cause relaxation of the abdominal wall without detectable contraction of the diaphragm.

In a preliminary report (5), it was stated that cervical vagotomy on the right side abolished most of the response to stimulation. Subsequent observations have shown that the effects of unilateral vagotomy depend upon the position of the animal. If it is lying on the vagotomized side the period of respiratory arrest is greatly prolonged and the response to stimulation on the upper side is markedly reduced or abolished. Reversing the position of the animal in the middle of a period of respiratory arrest, so that the vagotomized side is up results in increased sensitivity to the reflex or in the immediate resumption of spontaneous breathing. Alternate periods of inhibition and spontaneous breathing can be produced by this means with great regularity. The periods of spontaneous breathing are frequently characterized by an early slowing of rate apparently by a process of adaptation. Figures 2 and 3 illustrate the type of record obtained with unilateral vagotomy and unilateral pneumonectomy respectively. Thus the threshold to stimulation and the duration of inhibition for a

given dose of pentothal in a unilateral vagotomized animal depend upon which side the animal is lying. Bilateral vagotomy abolished the reflex response and further prolonged the period of inhibition. The effects of unilateral vagotomy appear to persist indefinitely in the recovered unanesthetized animal; for example, the average
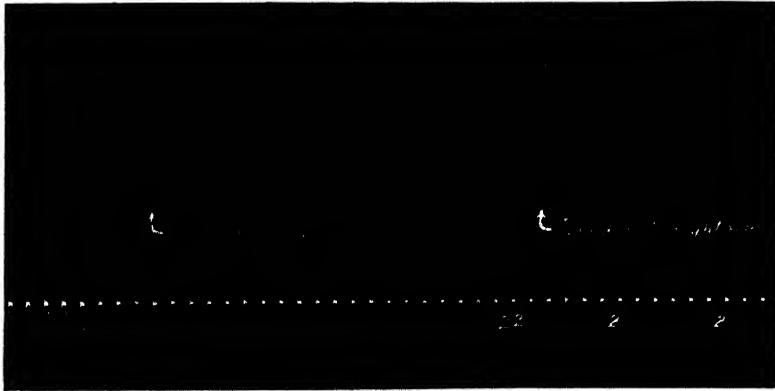
Fig. 2. RESPIRATION IN A DOG WITH A RIGHT UNILATERAL CERVICAL VAGOTOMY. The numbers under the time record are minutes since the last dose of nembutal (2.0 cc. of 2.5%). The state of anesthesia was such that the dog was not only in apnea but it was also refractory to 1 kg. stimulus when it was lying on its right side. On its left side, it breathed spontaneously.
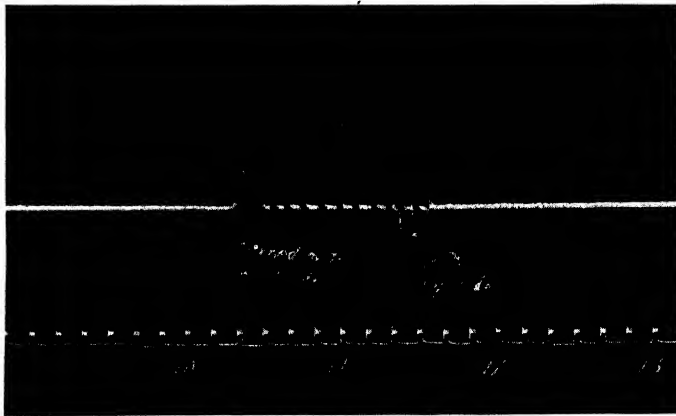
Fig. 3. RESPIRATION IN A DOG WITH LEFT-SIDED PNEUMONECTOMY. The numbers under the time record are minutes after the last dose of nembutal (1 cc. of 2.5%).

respiratory rate in a dog with left sided cervical vagotomy, about 4 months post operative, was 27 when lying on the right side, and 18 when lying on the left side.

The responses under deep pentothal anesthesia were studied in 6 dogs from two weeks to eight months following unilateral pneumonectomy. In each instance the pattern of response during pentothal inhibition followed that of unilateral cervical vagotomy, i.e., lying on the operated side depressed sensitivity to the reflex compared to the opposite position. The period of inhibition was also longer and spontaneous

breathing was slower.   These animals also showed a marked difference in respiratory rate with change in position when unanesthetized.   In a typical case, seven months postoperative, the rate was 21 when lying with the pneumonectomized side up compared to a rate of 15 when in the opposite position.

<div align="center">DISCUSSION</div>

None of the above results confirms the suggestion of Draper and Whitehead (4) that the reflex is elicited by stretching the rectus abdominis and scalenus medius muscles by applying pressure at the point of their common insertion.   Response to stimulation over a relatively large area of the thoracic wall and disappearance of response following bilateral vagotomy rather suggests that it is elicited by deformation of lung tissue exciting receptors of the Herring-Breuer reflex.   Much of the rhythmicity of normal respiration is ascribed to this mechanism.   The remarkable sensitivity of these receptors to deformation has been shown repeatedly.   Adrian (7) found that impulses in pulmonary afferent fibers varied rhythmically with the heart beat when there was compression of adjacent lung tissue.   Hammouda and Wilson (8) observed respiration in the anesthetized dog whose thorax had been enclosed in an air-tight box.   Under these conditions an increase in pressure on the outside of the thorax of only a few mm. Hg increased the respiratory rate.

That such a reflex could operate under deep anesthesia is indicated by observations of Whitteridge and Bülbring (9).   Indeed these investigators think that many of the respiratory effects of various anesthetics are due to specific changes in excitability of the pulmonary stretch receptors (10).   Further evidence for the ruggedness of this reflex is found in Adrian's report that discharge of pulmonary afferent fibers persisted as long as fifty minutes after cessation of circulation.

The responses to change in position described above may be explained tentatively in terms of lung deformation produced by the gravity shift of mediastinal structures such as the heart.   In the intact animal lying on its side, the application of pressure to the thoracic wall causes deformation of the underlying lung tissue.   The mediastinal structures, particularly the heart, tend to stretch the upper lung, but to compress the lung on the under side.   Presumably the balance between excitatory and inhibitory influences is upset by the application of a mechanical stimulus to the chest wall which reduces the degree of stretch of the lung on the upper side, but exaggerates the retraction of the lower lung.   Section of the vagus nerve on the upper side removes the inhibitory effects of the stretched lung allowing the excitatory effects of the lower lung to predominate.   When the position of the animal is reversed by placing the vagotomized side down, the excitatory impulses from the lower lung are removed and inhibitory influences of the upper side predominate.   A similar explanation may be applied to the results obtained with the unilateral pneumonectomized dog.   The attempts of Anderson and Lindsley to demonstrate unilateral effects from lung receptors on the intercostal muscles were unsuccessful (11).   Adrian however, reported changes in frequency of single afferent vagus fibers in response to change in position of the rabbit.

The results of the experiments reported here might indicate that, in the dog at least, a bilateral balance of opposing influences from the Herring-Breuer receptors

are normally acting upon the respiratory center. These effects are demonstrated by changing the position of the deeply anesthetized animal whose lung afferents have been sectioned on one side, or by observing the effect of position on the respiratory rate in the same animal when unanesthetized. The results also support the claim of Whitteridge and Bülbring that the Herring-Breuer reflex persists with deep anesthesia when other reflex mechanisms have been abolished.

Preliminary observations on man, following unilateral pneumonectomy, have shown little difference in respiratory rate with change in position during the post-operative convalescent period. Six out of nine patients examined had an average increase in rate of only 10 per cent when lying on the unoperated side. Two facts may account for this: the mediastinum is less flexible in man than in the dog and post-operative procedures to prevent mediastinal shift are commonly employed when pneumonectomy is performed in man. Change in position in one pneumonectomized patient under deep pentothal anesthesia produced slight changes in respiratory rate which were in the same direction as those observed in the experimental animal.

SUMMARY

The minimal pressure required for a reflex respiratory response during pentothal inhibition may be used to measure the rate of recovery of excitability. Reflex respiratory responses to light pressure on the chest wall in the deeply anesthetized dog are abolished by bilateral cervical vagotomy. Unilateral cervical vagotomy or unilateral pneumonectomy enhances the reflex response of the deeply anesthetized dog when lying with the operated side up; when the operated side is down the reflex response is depressed. The unilateral vagotomized or unilateral pneumonectomized dog has a faster respiratory rate when lying with the operated side up than in the opposite position. This difference in rate with change in position persists indefinitely in the unanesthetized animal.

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PROPRIOCEPTIVELY INDUCED REFLEX PATTERNS[1]

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LITTLE is known concerning the specific distribution of the peripheral effects of proprioceptive reflexes initiated by muscle stretch. A considerable portion of the available information is based on mechanical records of contraction. The present experiments were undertaken to determine by multiple electromyographic recording the response pattern in a selected group of arm muscles when one or several of them are stretched.

Lloyd (1) has conclusively demonstrated that the 2-neuron-arc myotatic reflex facilitates synergists of the muscle in which the afferent impulses arise and inhibits its antagonists. These effects are restricted to muscles acting at a single joint. Bineuronal reflex arcs, however, account for only a small fraction of the afferent fibers from skeletal muscle. Afferent impulses leading to multineuron reflex responses are almost certainly aroused by muscle stretch, for there is evidence of a spread of activity beyond the limits established by Lloyd for bineuronal reflexes. Denny-Brown (2) observed an excitation of the ankle extensor, soleus, in response to stretch of the knee extensor, quadriceps. Sherrington (3) demonstrated the response of certain contralateral limb muscles to stretch of the vastocrureus or the triceps surae.

Not only passive stretch, but also actively developed tension, results in proprioceptive reflex responses. These effects, too, extend beyond the limits of bineuronal representation established by Lloyd. For example, Cooper and Creed (4) showed that a proprioceptive reflex arising in the active tibialis anticus (upon stimulation of the appropriate ventral root) induced a contraction of the sartorius. Preventing the tibialis from shortening during its contraction, thus increasing its developed tension, resulted in a much stronger contraction of the sartorius. Gellhorn (5) has shown that proprioceptive reflexes arising in a muscle held under stretch during cortical stimulation augment the response (electromyograms of greater amplitude[2]) of its associated synergists at a neighboring joint, as well as its own.

Although Gay and Gellhorn have recently found that either passive stretch or actively developed tension of muscles (stimulation of a ventral root) excites the motor cortex (7), Gellhorn's observations show that the cortically induced response is altered only quantitatively and not qualitatively, by proprioceptive reflex facilitation. In other words, the *pattern* under the conditions of the experiments is determined by the site of cortical stimulation and modification of the response by proprioceptive reflexes is limited to alterations in the relative intensities of muscle response within the pattern. It may be assumed that if proprioceptive reflex activity is aroused in the absence of specific cortical stimulation the pattern of response will be a function of the spinal cord and not of the cortex. The following experiments show that proprioceptive impulses, set up by stretching a muscle, activate muscles in specific patterns similar to those found by Bosma and Gellhorn (8) to be elicited by cortical stimulation. These patterns include functionally associated muscles acting at neighboring joints.

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[2] It has been experimentally demonstrated that under cortical stimulation, E.M.G. amplitude is a reliable measure of the mechanical response (6).

METHOD

Cats and monkeys were anesthetized with Dial-urethane (Ciba)[3], 0.45 cc/kg., i.p. Potentials were led off by means of fine wires sewn into the muscles, amplified and recorded by an Offner crystograph. Drills were inserted in the humerus and ulna and fixation achieved by metal braces attached to the drills. Proprioceptive stimulation was accomplished either by passive movement of a joint or by a load applied to the tendon of a muscle. The biceps, triceps, flexor carpi (usually ulnaris) and extensor carpi (usually radialis) and the brachioradialis were routinely used.

RESULTS

Stretch of Elbow Extensors. In a sensitive preparation, a slight flexion of the elbow (e.g., reducing the angle from 90° to 75°) leads to excitation of the triceps and flexor carpi. The flexor carpi responds even after it has been tenotomized and, therefore, is presumably not affected mechanically by the elbow movement. Moreover,
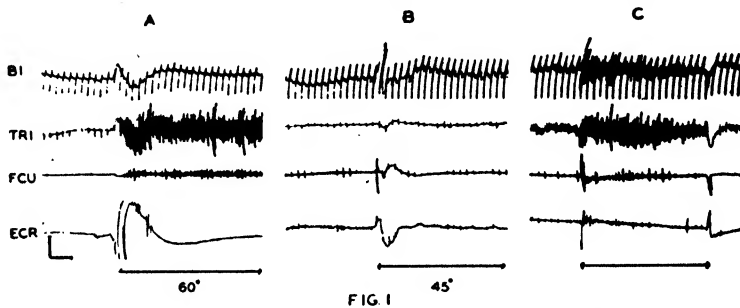
Fig. 1. RESPONSE TO ELBOW FLEXION before and after tenotomizing the triceps brachii (TRI) and the response to a pull on the triceps tendon. *Monkey.* A. The flexor carpi ulnaris (FCU) has been tenotomized. Elbow flexion excites the TRI-FCU complex. B. After triceps tenotomy, elbow flexion elicits no response. C. A pull on the triceps tendon excites the TRI-FCU complex. Co-contraction occurs in the biceps (BI), whose response is favored by an obtuse elbow angle (120°). Vertical axis = 60 μv. In all figures (read from left to right) the horizontal axis equals 1 sec.

the response in the flexor carpi is as great as the maximal response obtainable by a direct pull on its cut tendon and this involves a pull of several hundred grams.

Figure 1 illustrates the effect of tenotomizing the triceps on the reflex response to elbow flexion. Before tenotomy of the triceps, flexing the elbow to 60° resulted in good responses in both the triceps and flexor carpi. This result persisted after tenotomy of the flexor carpi (fig. 1A). After tenotomizing the triceps also, even greater flexion elicited no response in either muscle (fig. 1B) although both still responded to a pull on the triceps tendon (fig. 1C). This shows conclusively that proprioceptive impulses arising in the triceps as a result of its passive stretch reflexly excite the flexor carpi.

Figure 1C shows that a pull on the tenotomized triceps induced a distinct E.M.G. in the biceps, although the reflex induced previously in the same preparation by flexion of the elbow failed to do so (fig. 1A). It is suggested that the different

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[3] Kindly supplied by the Ciba Pharmaceutical Company.

behavior is due to the fact that the pull on the triceps tendon (fig. 1C) was exerted while the elbow was at an obtuse angle which is proprioceptively favorable to the biceps, whereas in figure 1A the triceps was proprioceptively excited through movement of the elbow to an acute angle which is unfavorable to the biceps. It was noted in an earlier study (5, 6) that proprioceptive reflexes in response to cortical stimulation occur preferentially in the stretched muscle. However, this induction of activity in the antagonist ('co-contraction') shows considerable individual differences which may in part be due to the degree of tonic activity (8). A rather strong co-contraction is illustrated in figure 2A. Flexing the elbow to 45° activates the triceps complex (TRI-FCU) although the flexor carpi is tenotomized and activates the biceps complex (BI-ECR-BR) to a lesser degree. That the activity of the latter is
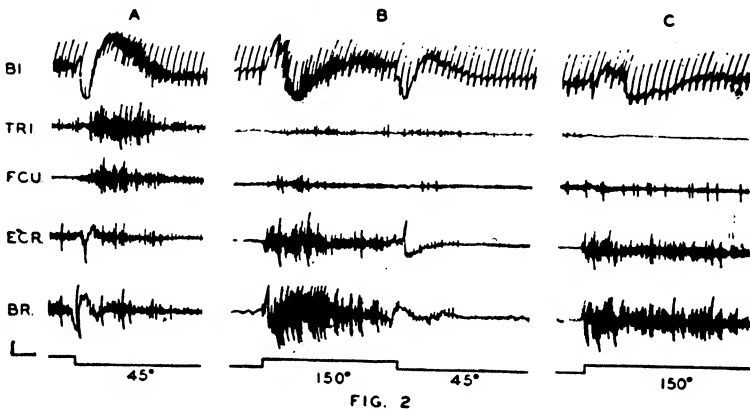
Fig. 2. RESPONSE TO ELBOW FLEXION AND EXTENSION before and after tenotomizing the triceps and biceps, respectively. *Monkey.* The flexor and extensor carpi were previously tenotomized. *A.* Flexing the elbow to 45° activates the TRI and the tenotomized FCU. Co-contraction occurs in the biceps complex. *B.* After tenotomy of TRI in addition to the carpal muscles. Extension of the elbow elicits the same response as was observed prior to the tenotomy of TRI, i.e. activation of the biceps (BI), brachioradialis (BR) and the tenotomized extensor carpi radialis (ECR). Flexion now activates nothing, showing that the stretch reflex arising in TRI in A was responsible for the excitation of FCU and the co-contraction of BI. *C.* After tenotomy of BI also, elbow extension does not activate the BI, and the response of the associated ECR and BR is reduced. Vertical axis = 300 microvolts in channel 2, and 30 microvolts in all other channels.

a co-contraction dependent on the proprioceptive triceps reflex is shown by the fact that, after tenotomy of the triceps has abolished its own response to elbow flexion, the biceps complex is also silent (fig. 2B, 45°). Summarizing our experience it may be said that the biceps and extensor carpi may be unaffected by elbow flexion, may exhibit a slight co-contraction, or may show an inhibition of tonic activity.

Stretch of Elbow Flexors. Extension of the elbow (fig. 2B) results in marked activity of the biceps, extensor carpi and brachioradialis. The response in extensor carpi radialis cannot be attributed to its being stretched by extension of the elbow, for its tendon had been cut. It therefore appears that its response is due to reflexes arising in other muscles which are stretched, such as the biceps and brachioradialis. The association of a response in the unstretched extensor carpi with one in the

stretched brachioradialis may be seen in figure 2C. The biceps had been tenot-omized, therefore neither responded nor contributed to the response of the other muscles. The lesser response in the 'triceps complex' (triceps-flexor carpi) is rather insignificant (fig. 2B) and is best explained as a slight co-contraction (8, 5).

A load on the biceps tendon likewise elicits a strong biceps-extensor carpi re-sponse (fig. 3). In this case the angle of the elbow is fixed and there can be no sus-picion of a direct mechanical effect on the extensor carpi.

Stretch of Wrist Extensors. In experiments with stimulation of the motor cortex, Gellhorn (5) found that the proprioceptive facilitation within a muscle complex may act in either direction, e.g., from biceps to extensor carpi, or the reverse. Apparently reflexes elicited by stretching a carpal muscle may induce similar reflex responses in specific upper arm muscles in the absence of cortical stimulation, since it was found
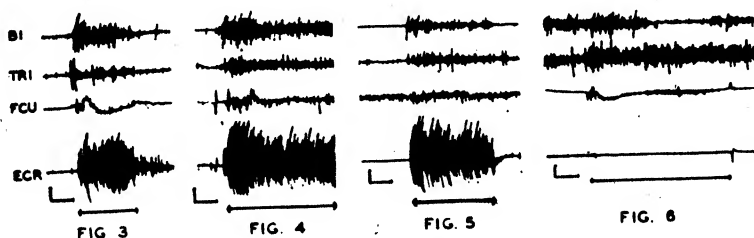
Fig. 3. RESPONSE TO A LOAD OF 500 G. ON THE BICEPS TENDON. *Cat*, all tendons intact. The response is primarily limited to the BI and its 'associated synergist' ECR. A lesser 'co-contraction' occurs in the antagonistic complex. Vertical axis = 100 μv.

Fig. 4. RESPONSE TO VOLAR FLEXION of the cat's wrist to 80°. The principal response is in the ECR and its 'associated synergist' BI. The lesser response in the antagonistic complex is designated co-contraction. Vertical axis = 100 μv.

Fig. 5. RESPONSE TO A = 500 G. LOAD ON AN EXTENSOR CARPI in the cat. The chief response is in the stretched ECR. Note that the response of the BI is initially strong, in contrast to the gradual development of the 'co-contraction' in the TRI. Vertical axis = 100 μv.

Fig. 6. RESPONSE TO A 500 = G. LOAD ON THE TENDON of the flexor carpi in the cat. Note the excitation of the TRI and FCU. The tonic acitivity in the BI shows inhibition, during which the TRI-FCU response is augmented. Vertical axis = 30 μv.

that volar flexion of the wrist excites the biceps-extensor carpi complex (fig. 4). The antagonistic complex may show co-contraction as in figure 4 or may be silent.

A load applied to an extensor carpi likewise excites the biceps. Figure 5, re-corded in the same cat as figure 4, shows that even though the stretch is limited to only one extensor carpi, the biceps response is good. It will be noted that in this record the triceps and flexor carpi were tonically active before the load was applied to the extensor carpi. This tonic activity resulted from fixation of the wrist at 180° extension. Despite this fixation in a position favoring the triceps-flexor carpi com-plex, stretch of the extensor carpi (load) yielded primarily a biceps-extensor carpi response. Although the triceps response is fairly strong, it is generated slowly in contrast to an initially strong response in biceps. There can be no reasonable doubt that the primary response is that of the biceps-extensor carpi complex and that the activity in the triceps-flexor carpi complex is a co-contraction.

Stretch of Wrist Flexors. Stretching the flexors carpi by extending the wrist

may result in a triceps-flexor carpi response as described in the preceding paragraph (fig. 5, extreme left). However, this cannot be consistently demonstrated, presumably because this degree of stretch of the wrist flexors does not always generate sufficient impulses. It can be demonstrated, however, that even though wrist extension fails to elicit a response in triceps, the latter's response to stimulation of an appropriate afferent nerve is facilitated (unpublished observations). This is in agreement with Gellhorn's observation of proprioceptive facilitation under conditions of cortical stimulation. Moreover, direct application of a load to the tendon of a flexor carpi regularly results in a triceps response. In the experiment of figure 6 this reaction appears as an augmentation of tonic activity in the triceps. The antagonistic complex may show nothing, a co-contraction, or an inhibition. In figure 6 the biceps shows initially a slight co-contraction, followed by inhibition during which the activity of both triceps and flexor carpi is slightly augmented.

DISCUSSION

This study has shown that reflexes of proprioceptive origin result in patterns of muscular coordination identical with those resulting from cortical stimulation. The myotatic reflex arising in a single muscle has been shown to excite not only this muscle and its synergists at the same joint, but 'associated synergists' acting at a neighboring joint as well. It was frequently noted that even greater activity could be evoked in a muscle by impulses arising proprioceptively in other muscles than by autogenous proprioceptive stimulation. Antagonists frequently show the inhibition expected from reciprocal innervation, but also frequently show co-contraction. This is not surprising, since fixation of a limb, when it involves the stretching of antigravity muscles as in the 'supporting reaction', leads to simultaneous contraction of flexors and extensors.

The complete agreement between the proprioceptive reflex patterns described in this paper and the modification of motor response through fixation in cortically induced movements suggests that the same mechanism is involved in both sets of experiments. Apparently postural changes (fixation of a joint, etc.) modify the effects of stimulation of the motor cortex through proprioceptive reflexes, but whether this modification is exclusively spinal or involves supraspinal mechanisms has not been investigated. Further experiments on the persistence of these specific reflex patterns of the stretch reflex after spinal transection, as well as the influence of deafferentation of a limb on the pattern of movement elicited by cortical stimulation, may clarify these questions.

Although these experiments have been restricted to a few muscles, the results suggest that any muscle of a limb is potentially subject to some regulation by proprioceptive impulses arising in all other muscles of that limb and that this mechanism is of great importance in muscle coordination no matter whether movements are elicited by reflex or by cortical mechanisms.

In view of these results, the observation that in certain cases of poliomyelitis a muscle which cannot be made to contract by voluntary effort may be excited by passively stretching another muscle, even an antagonist (9), or by a voluntary effort to contract other muscles (unpublished observations), appears less paradoxical. These

and related data (10) should be taken into consideration in a program of muscle reeducation.

SUMMARY

A study of proprioceptive reflexes is reported in which the effect of passive movements of the elbow and wrist joint or the action of pulling the tendon of an individual muscle is investigated by means of electromyograms. The experiments were performed on anesthetized cats and monkeys with the following results:

1. Passive movements of the elbow lead mainly to activity in the triceps and flexor carpi on flexion and to activity in the biceps and extensor carpi on extension. These effects persist even after tenotomy of the carpal muscles, but are abolished by tenotomy of the biceps and triceps. In the latter case the application of a load to the triceps again induces activity in the triceps complex (triceps-flexor carpi).

2. Stretching of wrist muscles by passive movements or by loading of an individual muscle induces activity in the upper arm muscles. Here again the effect is specific inasmuch as stretching of the flexor carpi induces activity in the triceps while loading the extensor carpi induces activity in the biceps.

3. If through passive movements or muscle stretch a considerable activity appears in the stretched muscle and in a specifically associated muscle (e.g., stretch of biceps causing activity in biceps-extensor carpi and stretch of triceps causing activity in the triceps-flexor carpi) the effect may also appear, but in lesser degree, in the antagonists of these muscles. This phenomenon is designated co-contraction.

4. It is emphasized that the same specific muscle patterns established earlier by Bosma and Gellhorn in experiments on stimulation of the motor cortex are produced by the myotatic reflex. The latter has a specific but less restricted effect than has been assumed heretofore.

5. Co-contraction as the result of the myotatic reflex shows the same features as seen in experiments with stimulation of the motor cortex.

6. The importance of this newer knowledge of the myotatic reflex for the problem of muscle reeducation is emphasized.

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RÔLE OF CARBON DIOXIDE AND OF THE HINDBRAIN IN AGENE-INDUCED CANINE EPILEPSY[1]

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NEWELL, Erickson, Gilson and Elvehjem (1) showed that dogs on a wheat gluten diet developed electroencephalographic patterns similar to those seen in human epilepsy. These changes could be detected as early as three days after the diet started and prior to the onset of clinical signs. In a previous paper (2), this group described the electroencephalographic changes as high voltage slow waves with spiking. When they were fortunate enough to get an animal to have a seizure during a recording of the EEG, the typical picture appeared. Mellanby (3) first noted that bread made from flour treated with nitrogen trichloride caused convulsions in dogs. Silver, Monahan, Klein and Pollock (4) demonstrated that wheat protein treated with varying amounts of nitrogen trichloride was the responsible agent for the abnormalities in the EEG. The first changes seen were an increase in the amplitude of the cerebral potential which fused with an increased frequency. A day later high voltage–low frequency waves appeared. This picture was well established by the end of three days of diet and continued throughout the course of the intoxication. There were sometimes spikes or spike and dome formations. Spontaneous seizures usually appeared after five days of diet, but if the amount of nitrogen trichloride added was increased, these seizures occurred earlier. The seizures were characterized by a 'slow build up' to a full discharge of the cerebrum. Then the characteristic iso-electric post-ictal period followed. Similar changes in the EEG have been observed in dogs fed gliadin, glutenin, lactalbumin, casein or amino acid mixtures when these had been treated with nitrogen trichloride (5).

In animals that have typical preconvulsive EEG's, convulsions can be induced by the inhalation of 20 per cent carbon dioxide and 80 per cent oxygen. The abnormalities so produced can first be detected from pick-up leads over the cerebellum.

EXPERIMENTAL METHODS

These experiments were conducted entirely on dogs that had been fed an adequate diet (6) containing flour treated with nitrogen trichloride (30–100 gm/100 lb.
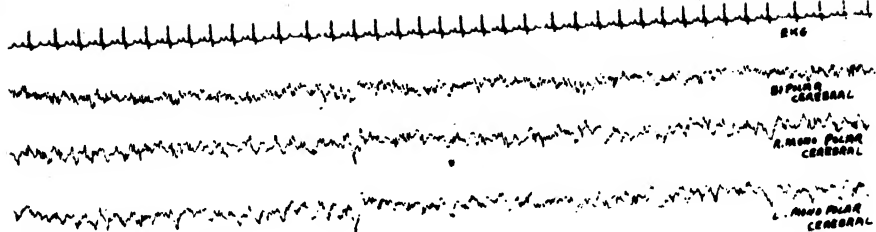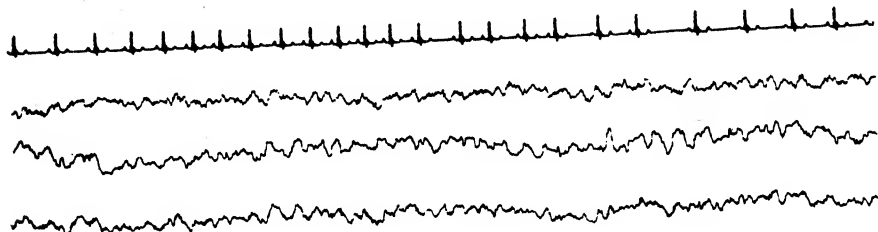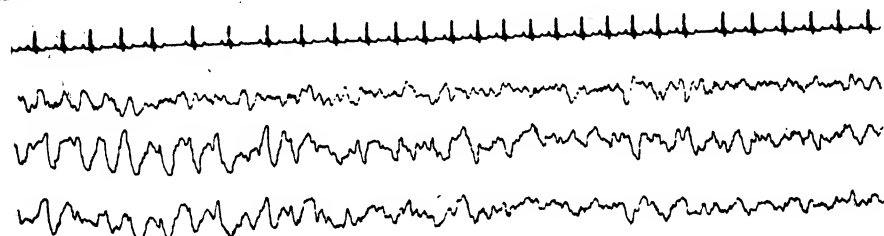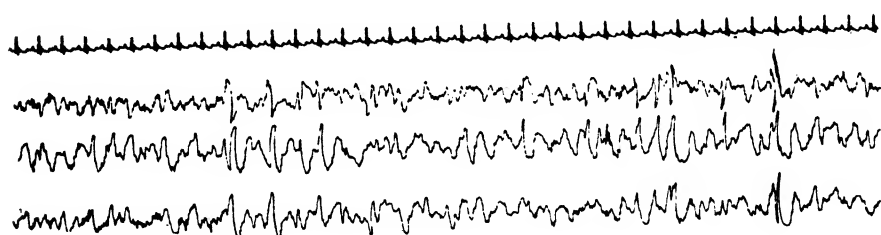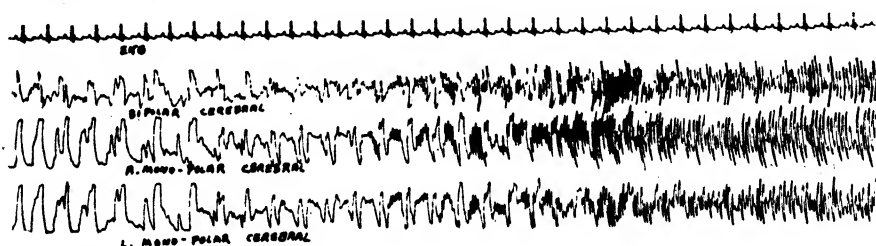
Fig. 1. *A*. Typical agene pre-CO₂ record. High voltage, slow waves with an occasional spike. *B*. Inhalation of 20% CO₂ plus 80% O₂ for 10 seconds. High voltage, slow frequency, begins to occur more frequently. EKG irregular and slower. *C*. Inhalation of gas mixture for one minute. Marked high voltage, slow frequency now. *D*. Inhalation of gas mixture for 2 minutes. Spikes now seen more frequently interspersed between high slow waves. *E*. Removal of CO₂ mixture after 3 minutes. Animal has seizure soon thereafter.

of flour). After several days' observation the animals were given the experimental diet. They were observed daily and, after various times during which known quantities of diet had been ingested, were used for acute terminal experiments.

A tracheal cannula was inserted and one femoral vein was exposed under ethyl ether anesthesia. Paralysis was induced with 25 mg. of dihydro-B-erythroidine hydrobromide[4] intravenously and artificial respiration started (50–75 cc/stroke, 10–15 strokes/min.). A continuous intravenous drip of a solution containing 1 mg/ml. of dihydro-B-erythroidine hydrobromide in 0.156 M sodium chloride was introduced into the exposed femoral vein. Electrodes were screwed into the skull overlying the right and left parietal cerebrum and over the cerebellar hemispheres. EKG was recorded from leads in the right fore and left hind limbs. The Grass six-channel electroencephalograph was used throughout. The mixture of carbon dioxide and oxygen used was commercially prepared. The respirator is so designed that a gas can be rapidly substituted for room air when desired.
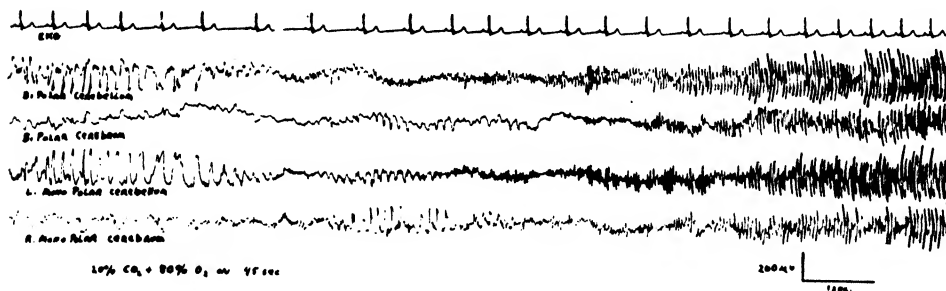
Fig. 2. TWENTY PER CENT CO$_2$ plus 80% O$_2$ inhaled for 45 seconds. Spikes and high slow waves first appear in cerebellar leads. Activity spread to cerebral leads. Seizure starts in cerebellar leads and after a short delay spreads to cerebrum. EKG is irregular and slow.

An initial period of about 30 minutes was allowed for stabilization of the electro-encephalographic record. Thereafter a mixture of 20 per cent carbon dioxide with 80 per cent oxygen was introduced into the respirator and the animals ventilated for 3 minutes. A 30-minute interval elapsed before the gas was again employed.

Control experiments were conducted on dogs fed an identical diet containing unagenized flour.

RESULTS

The typical abnormalities induced by ingestion of agenized (nitrogen trichloride treated) flour appeared first in the cerebellar leads and later in the cortical leads. They could be intensified or a seizure induced by the inhalation of the mixture (fig. 2). The fit usually appeared during the beginning or shortly after the end of the inhalation (fig. 1). Normal animals showed no seizures with inhalation of the mixture. However, they did show reversible changes; an increase in frequency and decrease of amplitude of the EEG and some slowing and irregularity of the EKG.

DISCUSSION

Silver (7) has reported definite pathology in the Purkinje layer of the cerebellum and the dentate nucleus of dogs fed flour treated with nitrogen trichloride. It is very possible that the activity of the hindbrain is a reflection of this pathology.

The synergistic action of carbon dioxide is as yet unexplained. Roseman, Goodwin and McCulloch (8) have shown that inhalation of 20 to 25 per cent carbon dioxide in oxygen results in a slowing of the heart rate and an increased frequency with decreased amplitude of the EEG in cats. They note that the oxygen tension of the cortex is also elevated. Dusser de Barenne, McCulloch and Nims (9) found that hypoventilation (increased carbon dioxide tension) shifts the pH of the cerebral cortex to the acid side. This low pH is associated with low electrical activity. Gibbs, Gibbs and Lennox (10) have shown that increasing the carbon dioxide tension of arterial blood causes a specific dilatation of the cerebral vascular bed with a resultant increase in the cerebral blood flow. It may well be that the increased blood flow through the brain results in a greater concentration of the toxic agent and this may be sufficient to intensify existing abnormality or precipitate actual seizure activity. Why this should occur when the gas mixture is initially administered or immediately after its removal is still unanswered. It may be due to rapid shifts in the brain pH or oxygen tension. The specific inhibitory effect of carbon dioxide on the cerebral cortex may allow subcortical structures activated by the toxic agent to manifest themselves. No definite interpretation is offered at present.

SUMMARY

Inhalation of 20 per cent carbon dioxide and 80 per cent oxygen is synergistic with the toxicity induced by the ingestion of flour treated with nitrogen trichloride. It accentuates existing electroencephalographic abnormalities and can precipitate a seizure in a susceptible animal. These are seen first in leads over the hindbrain and characteristically when the inhalation starts or shortly after it is terminated.

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ADAPTATION TO EXPERIMENTAL MOTION SICKNESS IN DOGS[1]

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SINCE 1942 the subject of experimental motion sickness has been studied extensively in this department. As only a few papers had been published on motion sickness in animals prior to this time (1, 2), it was essential to study the factors which influenced the susceptibility of the animal. It was found (3) that dogs were a more suitable species than cats since some 80 per cent were found to vomit after motion on a simple swing. By altering the extent of swinging it was possible to divide susceptible animals into three groups according to the ease with which they could be made to vomit. The most highly susceptible dogs were found to show an extremely consistent response to swinging even though they were used over a three- to four-year period. Of the different component motions of a swing none was as effective individually as the composite action. Changes in horizontal acceleration were the most effective stimuli, vertical motion being relatively ineffective. Animals which were susceptible to swinging wer. also found to become ill after driving in a motor truck and after exposure to motion in a small boat in rough water. Since the initial project was to find drugs which might serve to alleviate the symptoms of motion sickness, methods for assaying possible agents were worked out on dogs (4).

A large series of compounds, chiefly barbituric acid derivatives, were subsequently tested for their ability to prevent motion sickness in dogs (5). Many of these substances were found to possess this property which appeared to act specifically against motion sickness since this effect was not related to the hypnotic or anesthetic action of the compound. The response of human beings to treatment with a number of barbiturates was also tested against motion sickness produced by swinging (6). One compound V-12, ethyl β-methyl allyl thiobarbituric acid[2] was tested by various workers on sea trials and was found to be an effective therapeutic agent. The results of the Canadian trials with various drugs have been reviewed by Noble, Sellers and Best (7).

During the five years in which a colony of some 25 susceptible dogs was maintained for these various experiments, changes in susceptibility to swinging due to adaptation were noted. The results described in this paper are a summary of these

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observations and some additional experiments designed to obtain more information on the subject of adaptation to motion.

METHODS

Dogs of various types, ages and sex susceptible to motion were selected at random for these experiments. They were usually of from 5 to 10 kg. in weight. Except during the experiment they were housed in a separate building and had no contact with the swings. Each animal was fasted for 19 hours but immediately before swinging was fed a small meal of minced meat. Dogs which did not vomit following 45 minutes in the swing were considered to be immune or protected in therapeutic experiments. The swing used was electrically driven with a radius of $14\frac{1}{2}$ feet. It passed through an angle of 90° and had a frequency of 15 complete swings per minute. To determine different degrees of susceptibility the angle of swinging could be reduced. Dogs which vomited when the angle was reduced to $22\frac{1}{2}$° were considered highly susceptible while those which vomited when swung through an angle of 45° but not $22\frac{1}{2}$° were classed as moderately susceptible. In order to minimize adaptation, which was expected to occur, in early experiments animals were not used more frequently than once a week. For the last three years of the study, however, the dogs of the moderately and highly susceptible groups were used at intervals of every five days except during the usual holiday periods. In one experiment a dog was exposed to vertical motion, by a system of pulleys and ropes; the swing was used to drive the apparatus as previously described (3).

RESULTS

If one considers the effect of repeated exposures to motion on all of the dogs which have been observed over periods of years, there is usually a definite decrease in susceptibility of the animal. This adaptation effect probably occurs in every animal although the degree varies from an animal becoming totally immune to one which requires a slightly increased time of swinging before vomiting. Even when the dogs were used only at weekly intervals, some 30 per cent of the animals of the least susceptible group (i.e. those vomiting only when swung through an angle of 90°) over a two-year period become refractory to swinging. Others required longer periods of swinging before becoming ill. Such an effect rendered low susceptible animals of this type unsatisfactory for therapeutic experiments where a consistent control response was essential. Moderately or markedly susceptible dogs, even though used at intervals of only five days, seldom became immune to motion although some of them showed a decrease in susceptibility. The response of the majority of the susceptible animals remained practically unchanged over a number of years. One dog of this type was obtained in June 1943. The first 10 times the animal became ill it averaged 6 minutes on the swing. Three years later on 10 consecutive swingings at 5-day intervals the same dog averaged 10.5 minutes for its time of vomiting. Over this total period the animal vomited on 100 occasions and even though used for protective therapeutic experiments the average time of vomiting was 11.6 minutes.

Influence of the Interval between Swinging on Adaptation. A number of dogs

were swung at different intervals of time to test their susceptibility. These animals had been susceptible and showed a positive response to swinging for two to three years before these experiments were started. A brief protocol of the history of each animal is added to the tables. The effect of swinging at weekly intervals is shown in *dog 66* which had developed increasing adaptation to motion. Five other dogs required swinging at more frequent intervals to show this phenomenon. In these experiments the swing was stopped as soon as the dog vomited (table 1).

It is seen that the 5 dogs listed all showed adaptation to motion since the time taken to vomit and the incidence of vomiting tended to become gradually reduced. In the case of *dog 66*, swinging at weekly intervals was effective whereas *dogs 47* and *63* required repeated swinging twice weekly for a considerable period to develop resistance. The animals used daily or twice daily become immune rapidly. Such treatment apparently was cumulative and had a long lasting effect since the adaptation occurred gradually and was still present after rest intervals of 6 to 10 weeks. *Dog 27* became totally resistant and *dog 63* nearly so after the repeated exposures to motion. In three cases animals were compared at different occasions using different intervals between swinging. It can be noted that *dogs 25* and *63* became adapted much more rapidly when swung daily than when swung at 2- or $3\frac{1}{2}$-day intervals. Similarly *dog 67* showed a more rapid response when swung twice daily.

Influence of the Duration of Swinging on Adaptation. Three dogs were exposed to motion for a definite period of time, irrespective of whether or not they vomited, until they became adapted to motion. The results were then compared with those obtained after a suitable rest period by allowing the dogs to adapt as described in the preceding section. The time interval between swing tests in this case was kept constant. In many cases the animals became ill repeatedly (table 2). It would appear from those results that, in the case of *dog 60*, adaptation was accomplished more rapidly in the experiments where swinging was continued for 45 and 60 minutes, although in the other animals there was little difference. Unfortunately, the general tendency for immunity to develop, as noted previously, makes it difficult to assess changes in the same animal and, in many cases where swinging was stopped after vomiting, the times actually approximated 45 minutes. *Dog 42*, which was swung twice daily, showed a gradual increasing immunity so that at first vomiting occurred twice on the morning swing and once on the evening swing. As adaptation increased, vomiting took place only once on each time and then only in the morning until finally immunity was present.

Adaptation to a Different Type of Motion. One dog only was found to vomit consistently when exposed to vertical motion of a distance of eight feet four inches (double that occurring during ordinary swinging). It seemed of interest to test whether or not the adaptation to vertical motion would afford protection on the swing and vice versa. Experiments on this animal are listed in table 3. In two tests the animal was swung 45 minutes irrespective of whether vomiting occurred.

In the first test it may be seen that *dog 68* became adapted to the swing and remained so. However, on the sixth day it vomited in 35 minutes when exposed to vertical motion. In the third test the animal became immune to vertical motion after five daily runs and on the seventh day did not vomit when tested on the swing.

It seems therefore that adaptation to both types of motion was accomplished on one occasion but not on the other. The other tests on this animal shown in the table are typical of the increasing adaptation as previously described.

Adaptation under Drug Therapy. Some experiments were designed to see whether certain substances would inhibit or enhance the development of adaptation

TABLE I.   ADAPTATION TO MOTION AFTER SWINGING AT DIFFERENT INTERVALS OF TIME

Dog 66

DAYS	1	7	14	21
		Time before vomiting, min.		
Initial test	25	45	N	N
After 4-wk. rest	25	40	N	N
" 2 " "	14	N		
" 2 " "	19	N		

Dog 25

DAYS	1	2	3	4	5	6	7	8	9
				Time before vomiting, min.					
Initial test	40	40	N	N					
After 4-wk. rest	40		35		40		N		N

Dog 27

Initial Test	40	N	30	N	35	N	N	N	
After 3-wk. rest	25	N	N						
" 6 " "	N	N							
" 10 " "	N	N							

Dog 67

DAYS	1	1	2	2	3	4	5	6
			Time before vomiting, min.					
Initial test	40	40	N	N	N		N	N
After 10-wk. rest	20	—	25	—	40	40	N	N

Dog 47

DAYS	1	3	7	10	14	17	21	24	28	31
					Time before vomiting, min.					
Initial test	22	22	33	15	33	N	43	N	35	N·
" "	18	19	19	25	19	20	22	24	25	25

Dog 63

DAYS	35	38	42	45	49	52	56	59	63	66
Initial test	N	N	35	N	37	N	N	N	N	N
" "	22	32	N	N	36	30	30	42	N	N

Dog 63 (cont'd)

DAYS	1	2	3	4	5	6
After 2 wk. rest	25	30	30	40	N	N
" 3 " "	35	N	N			
" 8 " "	N	N				
" 10 " "	40	N	N			

N = not ill in 45 min.

Dog 66 Moderately susceptible for previous 2 yrs., changing to low susceptibility before start of above experiment.

Dog 25 After 2 yrs. of testing had changed from moderate to low susceptibility; above experiment 1 yr. later.

Dog 27 Moderately susceptible throughout, used for 3 yrs. previous.

Dog 67 After 1 yr. of testing had changed from moderate to low susceptibility; above experiment 7 mo. later.

Dog 47 After 9 mo. of testing had changed from moderate to low susceptibility; experiment 11 mo. later.   *Dog 63* Moderately susceptible throughout; used for 2 yrs. previous.

to motion and in one case an animal was treated with V-12 to see whether or not immunity would develop even though the symptoms of motion sickness were prevented. *Dog 47*, swung daily, also received .5 cc. of prostigimine 1 in 4000 subcutaneously. Adaptation occurred in the same time as in a previous test where no therapy was given. *Dog 44* received daily 20 mg./kg. orally of allyl (1-methyl butyl) imino thiobarbituric acid, a substance which appeared to increase the susceptibility of dogs to motion sickness. Adaptation under such treatment took place in the expected normal fashion. *Dog 63* received daily injections of 0.4 mg. hyoscine hydrobromide and was swung daily. Immunity developed as in control tests, such treatment apparently neither increasing nor preventing adaptation.

Dog 47 was treated daily for 11 days with 10 mg./kg. orally of V-12; during this period the animal was swung twice weekly. Such treatment prevented vomiting and all symptoms of sickness on each occasion and immunity had developed when therapy was stopped. The same animal was similarly treated for 11 days with V-12 after it was adapted and swinging continued. After cessation of treatment the animal was still immune even though swinging was continued for 65 minutes. Apparently effective therapy with V-12 did not prevent adaptation from taking place nor did it interfere with its continuation after it was once established.

Conditioned Reflex to Motion. In initial experiments it was believed that dogs susceptible to motion might readily become conditioned so that they would vomit at the sight of the swing or the container in which they were secured to it. When animals were swung every seven or five days even though this was continued over a period of years, no case of conditioning was ever encountered. In the 10 animals which were used to demonstrate adaptation, there was also no sign of conditioning even though some dogs were swung twice daily. In all cases the trend was for animals to become refractory to swinging rather than the reverse. Recently Dr. W. H. Johnston of the Zoology Department of this University made observations on a dog susceptible to motion which had become conditioned to the swing so that at the sight of it salivation and vomiting would occur. This animal was treated with V-12 and

TABLE 2. ADAPTATION TO MOTION AFTER SWINGING FOR DIFFERENT LENGTHS OF TIME

Dog 60

DURATION OF SWINGING, MIN.	1 DAY	3 DAYS	7 DAYS	10 DAYS	14 DAYS	17 DAYS	21 DAYS	24 DAYS	28 DAYS
				Time before vomiting, min.					
Until vomiting.............	20	15	15	15	13 / 10	35	N	N	
45.....................	30	N	30	N	{ 25, 32 }	N	20	N	N
60.....................	{ 32, 35, 60 }	N	58	N	N				
Until vomiting—after 8-wk. rest..............	35	35	N	N					
Until vomiting—after 10-wk. rest..............	N	N							

Dog 44

	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	8 DAYS	9 DAYS	10 DAYS
					Time before vomiting, min.					
45.....................	25	N	45	38	18	35	{ 25, 42 }	25	N	N
45—after 3-wk. rest.......	40	30	15	25	N	N				
Until vomiting—after 6-wk. rest..............	40	45	35	N	N					
Until vomiting—after 10-wk. rest..............	26	25	25	23	23	25	30	N	N	

Dog 42

	1 DAY	1 DAY	2 DAYS	2 DAYS	3 DAYS	3 DAYS	4 DAYS	4 DAYS	5 DAYS	5 DAYS	6 DAYS	7 DAYS
						Time before vomiting, min.							
45.....................	{ 20, 35 }	40	{ 30, 30, 40 }	N	20	N	20	45	40	N	N	
45—after 3-wk. rest.......	30		35		40		25		N			
Until vomiting—after 6-wk. rest..............	40		45		35		N		45		N	N
Until vomiting—after 10-wk. rest..............	35		N		N							

N = not ill in 45 min. or time indicated.

Dog 60 After 6 mo. of testing had changed from moderate to low susceptibility; above experiment 18 mo. later. *Dog 44* Moderately susceptible throughout; used for 22 mo. previous.

Dog 42 After 1 yr. of testing had changed from moderate to low susceptibility; above experiment 10 mo. later.

curiously enough its symptoms due to conditioning were prevented as was vomiting after swinging. Whether this animal could be made to become adapted to motion

while being swung under V-12 therapy would be of interest since one might expect unconditioning to occur under these circumstances.

TABLE 3. ADAPTATION TO SWINGING AND VERTICAL MOTION

	Dog 68							
DURATION OF SWINGING, MIN.	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	8 DAYS
	Time before vomiting, min.							
45....................	(20)	40 {	18} 30}	N	N	(35)	N	N
45—after 3-wk. rest.......	40	35	40	N	40	N	N	
45— " 6 " "	17	(10)	(N)	(35)	(N)	(N)	N	
Until vomiting—after 10-wk. rest..............	21	25	30	30	N	N		

N = not ill in 45 min. Brackets indicate that vertical motion was used.

Dog 68 Highly susceptible at start for 5 mo., moderately susceptible for 9 mo., low susceptibility for 6 mo. before experiment.

DISCUSSION

The results reported in this paper show that the dog may readily become adapted to motion sickness. Over a period of years during which a colony of susceptible animals was used for repeated tests there was a gradual change so that the susceptibility diminished. This was shown in many cases by an increased degree of swinging necessary to make the dog vomit. Animals which showed the lowest susceptibility at the start become immune to motion much more rapidly than those of highest susceptibility. Of this latter group the response of some animals remained practically unchanged when swung every five days for as long as three years. The gradual alteration in susceptibility which takes place in dogs is of particular importance when one is conducting therapeutic experiments. Besides an alteration in the control test, animals as they become less susceptible to motion are effectively treated by progressively smaller doses of drugs (4). The details of experiments to show adaptation have been given in the accompanying tables. The 10 dogs shown all became relatively resistant to swinging after repeated exposures. In one case with intervals of one week between tests the animal became refractory. In general the shorter the interval between tests the more rapidly did adaptation take place. Even with rest intervals up to 10 weeks between tests, however, the animals all showed a progressive loss of sensitivity. Some tests were designed to see if prolonged swinging on each occasion would enhance the onset of refractiveness. In one case this seemed to be so but the changing susceptibility of the dogs to repeated tests made the observations unreliable.

In the human being adaptation to any one form of motion appears to be highly specific in that individuals became ill when exposed to other forms of motion (8). One dog which was normally susceptible to vertical motion remained so although it was made refractory to the ordinary swing. On the other hand when it was made immune to vertical motion it was found to be also immune to swinging. Apparently

these two types of motion were sufficiently similar so that adaptation was effective to some extent in both.   Various drugs were administered to see if they would affect the development of resistance in any way.   Two substances, prostigimine and a thiobarbiturate, which might be expected to render the dogs more susceptible to motion sickness had no effect on adaptation to motion.   Hyoscine, which is effective in treating motion sickness in human beings but not in dogs, did not enhance or prevent the onset of adaptation.   The barbiturate V-12 was used to prevent motion sickness in a susceptible dog and this animal was able to become adapted to repeated swings even though it was not ill.   Also the refractory state was maintained by swinging when the animal was still protected by treatment.   This experiment would suggest that effective therapy of motion sickness is compatible with the development of adaptation to motion.   Throughout the experiments on repeated exposure to motion no animal showed any evidence of becoming conditioned to vomiting.   One such animal is reported from a different laboratory.   The salivation and vomiting which normally took place when this dog was simply placed on the swing was prevented with V-12 as was any effect of actual swinging.

SUMMARY

Dogs which have been used for repeated tests on motion sickness show a gradual adaptation to motion on a swing.   This refractoriness varies in extent, but is most readily produced in dogs having low initial susceptibility to motion.   On the other hand, highly susceptible animals may show only little change to swinging every five days over a three-year period.

Although swinging every seven days may cause adaptation, the state rapidly follows daily or twice daily swingings.   The speed of development is apparently proportional to the number of exposures.   Some evidence is presented that adaptation occurs more rapidly when the duration of swinging is increased.   Treatment with prostigimine or hyoscine did not inhibit or enhance adaptation to motion. Adaptation to motion took place and was maintained even though a dog was effectively treated by the barbiturate V-12.   Conditioned vomiting to the swing was not observed.   One animal of another worker showed this reaction which was prevented by treatment with V-12.

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EFFECT OF ELECTRICAL STIMULATION UPON ATROPHY OF PARTIALLY DENERVATED SKELETAL MUSCLE OF THE RAT[1]

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ALTHOUGH considerable experimental data have been accumulated concerning the effects of electrical stimulation upon the atrophy of totally denervated skeletal muscle (1–8), there is no direct information of the behavior of partially denervated muscles toward such treatment. And yet, in its clinical applications, electrotherapy of paralyzed muscles involves muscles whose denervation is often incomplete. The present studies were undertaken to obtain such experimental evidence.

METHODS AND MATERIALS

The animals used throughout these experiments were adult male albino rats weighing from 275 to 350 gm. The muscle investigated in all cases was the gastrocnemius. In the rat, this muscle receives its major motor innervation from the fourth and fifth lumbar nerves with a minor contribution from the sixth lumbar nerve (9, 10).

By selective bilateral lumbar nerve section three degrees of denervation of the gastrocnemius were produced: moderate, severe and complete. At the end of the experimental period supra-maximal electrical stimuli were applied to the sciatic nerve (indirect stimulation) and to the gastrocnemius directly (direct stimulation) and the tension developed by the left and right muscles upon such direct and indirect stimulation was measured. The animals were then killed and wet weights of the muscle obtained. Tension measurements were made by a torsion myograph of the Blix type. Among those rats selected for treatment, the gastrocnemius on one side was stimulated once daily for the entire period of denervation with a modulated sinusoidal current having a carrier frequency of 25 cycles per second. The carrier frequency was sinusoidally modulated at a rate of 25 per minute. The stimulating electrodes were applied directly to the skin overlying the gastrocnemius.

The procedure for the daily stimulation was as follows: A 30-second period of stimulation followed by a 10-minute rest period and an additional 30-second period of electrical exercise. The animals were divided into the following experimental groups:

Group 1. Moderate partial denervation. Bilateral section of the fifth lumbar nerve.
a) 21 rats. No treatment. Sacrificed 14 days following nerve section.

TABLE 1. MUSCLE WEIGHTS OF UNTREATED GROUPS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	MEAN WT. OF GASTROCNEMIUS		CORRELATION COEFF.	T RATIO
			Left	Right		
		days	*gm.*	*gm.*		
5th...................	21	14	1.154	1.205	.78	1.98
5th...................	16	28	1.101	1.119	.01	.166
4th, 5th..............	26	14	1.120	1.154	.79	1.21
4th, 5th, 6th..........	22	14	.893	.885	.57	.228

TABLE 2. MUSCLE TENSIONS OF UNTREATED GROUPS, IN GRAMS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	DIRECT STIMULATION (THROUGH MUSCLE)				INDIRECT STIMULATION (THROUGH NERVE)			
			Left	Right	Correlation coeff.	T ratio	Left	Right	Correlation coeff.	T ratio
		days								
5th.................	17	14	1141.7	1309.8	.80	2.09	667.4	797.2	.75	2.02
5th.................	14	28	1057.1	1060.7	.24	.018	710.7	675.0	.27	.212
4th, 5th...........	22	14	992.3	1019.3	.63	.502	336.0	304.8	.68	1.58
4th, 5th, 6th.......	21	14	814.2	763.6	.60	2.04				

TABLE 3. MUSCLE WEIGHTS OF TREATED GROUPS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	MEAN WT. OF GASTROCNEMIUS		% DIFFERENCE	T RATIO
			Stimulated muscle	Untreated muscle		
		days	*gm.*	*gm.*		
5th...................	53	14	1.204	1.126	6.9	3.25[1]
5th...................	19	28	1.165	1.021	14.1[3]	2.57[2]
4th, 5th..............	24	14	1.157	.977	18.4[3]	5.81[1]
4th, 5th, 6th..........	25	14	1.132	.871	29.9[3]	10.4[1]

[1] Significant at the 1% level.   [2] Significant at the 2% level.   [3] These differences are significantly greater than that of the 5th nerve, 14-day group.

TABLE 4. MUSCLE TENSIONS OF TREATED GROUPS, IN GRAMS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	DIRECT STIMULATION (THROUGH MUSCLE)				INDIRECT STIMULATION (THROUGH NERVE)			
			Stimulated muscle	Untreated muscle	% difference	T ratio	Stimulated muscle	Untreated muscle	% difference	T ratio
		days								
5th..............	50	14	1255.1	1165.3	7.7	2.43[1]	727.3	722.3	0.7	.081
5th..............	19	28	1156.3	847.3	36.5[3]	3.65[2]	779.0	712.6	9.3	.758
4th, 5th..........	19	14	874.4	680.3	28.5[3]	3.09[2]	210.7	258.3	22.6	1.45
4th, 5th, 6th.......	25	14	1043.7	780.2	33.8[3]	8.6[2]				

[1] Significant at 2% level.   [2] Significant at 1% level.   [3] These differences are significantly greater than that of the 5th nerve, 14-day group.

b) 16 rats. No treatment.   Sacrificed 28 days following nerve sections.

c) 53 rats.   Electrical stimulation of one gastrocnemius.   Sacrificed 14 days following nerve section.

d) 19 rats.   Electrical stimulation of one gastrocnemius.   Sacrificed 28 days following nerve section.

Group 2. Severe partial denervation.   Bilateral section of fourth and fifth lumbar nerves.

a) 26 rats. No treatment.   Sacrificed 14 days following nerve section.

b) 24 rats.   Electrical stimulation of one gastrocnemius.   Sacrificed 14 days following nerve section.

Group 3. Complete denervation.   Bilateral section of fourth, fifth and sixth lumbar nerves.

a) 22 rats. No treatment.   Sacrificed 14 days following nerve section.

b) 25 rats.   Electrical stimulation of one gastrocnemius.   Sacrificed 14 days following nerve section.

Statistical significance was determined by the method of paired comparisons.

RESULTS

Weight and Strength of Untreated, Control Groups. An examination of tables 1 and 2 reveals that there are no significant differences in either wet weights or in muscle tensions through direct and indirect (nerve) stimulation between the left and right gastrocnemii for any of these groups.   With the exception of the 28-day group, a fair correlation of weight and strength loss exists between the muscle pairs.   Thus, in most instances following bilateral section of corresponding spinal nerves, both gastrocnemii of the rat undergo essentially the same degree of atrophy.   The application of an effective therapeutic agent to one of the muscles should then yield statistically significant differences in weight and strength between the muscles.

Muscle Weight and Strength of Treated Groups. In all these groups, daily electrical stimulation significantly retarded the loss of weight and strength of the treated muscle as compared to its paired untreated control (tables 3 and 4).   These differences although small in the case of the 14-day group with fifth lumbar nerve section become significantly greater as either the denervation is made more complete or its duration prolonged (table 4).

In no case was there any significant difference in muscle tension between treated and untreated muscles when tension development was elicited by indirect (nerve) stimulation.   The greater strength and presumably the greater mass of the treated muscles must be due to the effects of electrical exercise upon those contractile units which have lost their innervation.   That portion of the muscle with intact innervation is apparently unaffected by the regimen of electrical stimulation.

DISCUSSION

The results reported here conclusively demonstrate that, in the rat, electrical stimulation significantly retards the atrophy which a muscle undergoes following a partial loss of its motor innervation as well as that resulting from a complete denervation.

It is apparent, however, that the loss of motor innervation must be of considerable severity or duration before the weight and strength difference between treated and untreated muscles are sufficiently great to justify the use of electrical stimulation as a therapeutic agent in paralysis of skeletal muscle.

Thus, the increased weight and strength of the stimulated muscles when only the fifth lumbar nerves are sectioned for a period of 14 days, although statistically significant (t = 3.25, 2.43), are not particularly striking. On the other hand, when either the existing denervation is maintained for a longer period (28 days) or the motor innervation is subjected to a still further reduction, considerable and, presumably, important differences occur in the mass and contractile power of the muscles.

Since electrical stimulation does not significantly alter the tension responses of those parts of the muscle which still have a motor nerve supply, it is reasonable to conclude that: 1) the beneficial effects of electrical stimulation result from its action upon the denervated muscle fibers and 2) electrical stimulation, per se, does not have any deleterious effect upon those fibers with an intact innervation.

SUMMARY

1. Electrical stimulation by means of a modulated sinusoidal current with a carrier frequency of 25 cycles per second significantly retards the weight and strength loss of the gastrocnemius muscle of the rat which occurs following partial as well as complete motor denervation.

2. The differences in weight and strength between treated and untreated muscles, which have been subjected to a partial denervation, become significantly greater as either the period of denervation or the extent of the denervation is increased.

3. The difference in strength (and presumably weight) between treated and untreated muscles is due to the effect of the electrical stimulation upon those fibers which have lost their innervation.

4. The tension developed by those fibers whose innervation is still intact is apparently unaffected by daily electrical stimulation for periods of 14 to 28 days.

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DISSOCIATION OF POTASSIUM AND ACETYLCHOLINE SENSITIVITY OF FROG MUSCLE PRODUCED BY ISOTONIC GLUCOSE

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MANY pharmacological agents are known to modify the reaction of striated muscle to potassium and acetylcholine. For example, veratrine and thiocyanate markedly increase the sensitivity of muscular tissue to potassium and increase its sensitivity to acetylcholine to a lesser extent (1–3). Physostigmine, on the other hand potentiates the action of acetylcholine more than that of potassium (4, 6). Some substances may even produce a dissociation in the sensitivity of the muscle, with an increased response to acetylcholine and a decreased response to potassium. These latter cases of complete dissociation are relatively rare and their interpretation is difficult. From the results reported by Torda and Wolff (4–6), it appears that the combination of decreased acetylcholine sensitivity and increased potassium sensitivity is very unusual.

In the course of a study of the 'veratrinic' action of sodium thiocyanate (3), we have observed a striking example of this type of dissociation, which was regularly produced by isotonic glucose solution and seemed worth reporting.

METHOD

Ten frogs (Rana pipiens) were used in this study. After pithing the animal, the rectus abdominis was excised and divided along the median line. Each half-muscle was used in a separate experiment. Many tests were performed on the same preparation.

After excision the muscles were soaked in Ringer's Solution and kept in the refrigerator for one to four hours. The Ringer's Solution used throughout the experiments had the following composition: NaCl 0.6 per cent; KCl 0.0075 per cent; $CaCl_2$ 0.01 per cent; $NaHCO_3$ 0.1 per cent; distilled water.

When ready for use, each muscle was suspended in a muscle chamber containing 10 cc. of oxygenated Ringer's Solution at room temperature. The length of the muscle was isotonically recorded on a kymograph by means of a muscle lever and an ink writing pen.

Shortening was induced by injection into the bath of varying amounts of potassium chloride solution (1–2%) or acetylcholine[2] (5–10 mg/100 cc). The muscle was

then exposed to an isotonic solution of glucose for 1 to 15 minutes and the same substances were tested again. A third test was performed after the muscle had been washed with Ringer's Solution.
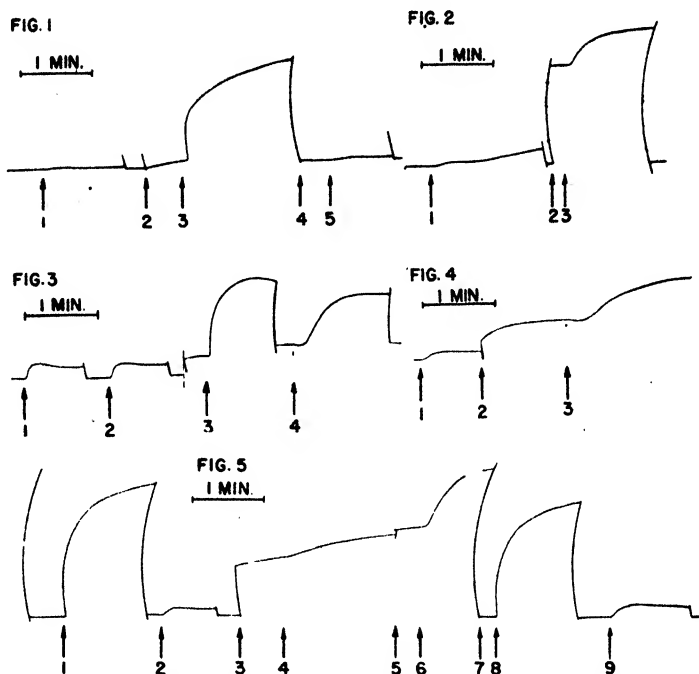
Fig. 1. *1.* Injection into the bath of 0.5 cc KCl 2%—muscle in Ringer's Solution. *2.* Recording started 30 sec. after replacement of Ringer's Solution by isotonic glucose. *3.* 0.5 cc KCl 2%—muscle in glucose. *4.* Ringer's Solution for 3 minutes. *5.* 0.5 cc KCl 2%—muscle in Ringer's Solution.

Fig. 2. *1.* 0.5 cc KCl 2%—muscle in Ringer's Solution. *2.* Isotonic glucose in 15 min. *3.* 0.5 cc KCl 2%—muscle in glucose.

Fig. 3. *1.* 0.5 cc KCl 2%—muscle in Ringer's Solution. *2.* 0.5 cc KCl 2%—muscle in Ringer's Solution. *3.* 0.5 cc KCl 2%—muscle in glucose for 2 min. *4.* 0.5 cc KCl 2%—muscle in glucose for 6 min.

Fig. 4. *1.* 0.5 cc KCl 2%—muscle in Ringer's Solution. *2.* Isotonic glucose for 3 minutes. *3.* 0.5 cc KCl 2%—muscle in glucose.

Fig. 5. *1.* 0.25 cc acetylcholine (10 mg/100 cc.)—muscle in Ringer's Solution. *2.* 0.5 cc KCl 2%—muscle in Ringer's Solution. *3.* Isotonic glucose for 3 min. *4.* 0.25 cc acetylcholine—muscle in glucose. *5.* Isotonic glucose for 1 min. *6.* 0.5 cc KCl 2%—muscle in glucose. *7.* Ringer's Solution for 5 minutes. *8.* 0.25 cc acetylcholine—muscle in Ringer's Solution. *9.* 0.5 cc KCl 2%—muscle in Ringer's Solution.

RESULTS

A few seconds after the rectus has been in contact with the isotonic glucose, it usually develops a spontaneous shortening which varies in its intensity from muscle to muscle. In most cases the degree of this glucose contracture remains small or moderate and does not disturb the course of the experiment. It soon reaches a steady level or at most increases so slowly that the effect of the substances tested can be evaluated with a fair approximation.

During this sugar contracture the acetylcholine sensitivity is markedly decreased and continues to decrease as long as the muscle remains in contact with the isotonic glucose solution. This rapid loss of acetylcholine sensitivity justified the term: sugar non-irritability (7). The sugar contracture disappears and the action of acetylcholine returns to normal shortly after the solution of glucose is replaced by normal Ringer's Solution. In our experiments the muscle was never allowed to remain in contact with the glucose for more than 15 minutes and the complete reversibility of the phenomenon was always easily demonstrated.

In striking contrast to this effect of isotonic glucose, the potassium sensitivity of the muscle is considerably increased during the sugar contracture. The intensity of the sensitization is comparable to the action of a powerful sensitizer to potassium such as sodium thiocyanate. In spite of the sugar contracture already present in most cases, the effect of the same dose of potassium chloride can be multiplied by five, ten or more times (fig. 1). The shortening induced by potassium under these conditions starts suddenly and rapidly reaches a high level. This effect, however, is not immediate. In most cases the injection is followed by a relatively long latent period before the shortening starts. In some preparations it was possible to record a small but definite relaxation of the muscle following the injection and preceding the potassium-induced shortening which, in these cases, was apparently less marked and less rapid than usual (figs. 3 & 4).

The sensitization to the potassium ion is very marked and perhaps maximal after the muscle has been soaked in isotonic glucose for about one minute (fig. 1). The reaction to potassium seems to decrease as the exposure to glucose is prolonged. Part of this may be an illusion due to the progressive development of the sugar contracture which makes exact quantitative comparisons difficult. After 15 minutes of contact, the effect of potassium is still definitely greater than normal, even when it is superimposed on a marked sugar contracture (fig. 2).

The increased potassium sensitivity observed under these circumstances is also immediately reversed by replacing the potassium with Ringer's Solution (fig. 1).

The opposite changes on potassium and acetylcholine sensitivity of the rectus produced by isotonic glucose are particularly striking when acetylcholine and potassium chloride are tested successively on the same muscle. If the respective concentrations have been chosen in order to produce a much greater initial effect by acetylcholine than by potassium in Ringer's Solution, the ratio is reversed under the influence of the glucose solution and the effect of potassium chloride becomes predominant (fig. 5). The muscle recovers its normal properties very shortly after washing with normal Ringer's Solution.

DISCUSSION

The effects of isotonic sugar solution on different frog muscles have been listed by Fenn (7): reversible non-irritability, slight contracture, increase in oxygen consumption, loss of electrolytes by diffusion (chiefly potassium and phosphoric acid), increased lactic acid concentration and an electrical change in the muscle which is temporarily negative but predominantly positive. Previous treatment with sugar prevents the contracture and the rise in oxygen consumption due to potassium (7),

but exposure to glucose was much more prolonged in such experiments than in the present ones in which we were concerned exclusively with the early effects of isotonic glucose.

Our findings confirm Fenn's description of the 'sugar contracture'. They also demonstrate a peculiar dissociation of potassium and acetylcholine sensitivity occurring very shortly after the exposure of the muscle to isotonic glucose. The action of potassium chloride is markedly increased. Some of our results, however, suggest that an antagonism between the action of glucose and potassium can be observed even in this early period; this is apparent in the long latent period following the injection of potassium chloride and especially for the temporary relaxation which is sometimes observed as a first effect of the injection. These are probably transitional phenomena which precede the effects reported by Fenn (7).

The opposite changes in potassium and acetylcholine sensitivity described in this report find no simple explanation. A loss of potassium from muscle decreases its sensitivity to potassium. Lactic acid increases it, but does not affect the acetylcholine sensitivity (4). Calcium-free Ringer's Solution increases the response to potassium but the effect of acetylcholine is decreased significantly only after long exposure (4).

The rôle of the sodium ion is probably predominant: muscles presenting the sugar non-irritability recover when again immersed in Ringer's Solution or any non-toxic solution containing sodium ions (8). Further, we did not observe any increase in potassium sensitivity when isotonic sodium chloride was used instead of isotonic glucose. It is probable that the changes in acetylcholine and potassium sensitivity are related to an ionic disturbance produced by isotonic glucose and involving the sodium ion. Their real mechanism, however, is still undetermined.

SUMMARY

Early effects of isotonic glucose on the frog's rectus abdominis include a small or moderate contracture, a decrease of acetylcholine sensitivity and a marked increase in potassium sensitivity. These changes are easily reversible. The increased reaction to potassium is often preceded by a relatively long latent period and sometimes by a slight relaxation of the muscle. The mechanism of these phenomena is not established.

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PLASMA PROTEIN CONCENTRATIONS AND ORGAN WEIGHTS OF CASTRATED AND TESTOSTERONE PROPIONATE TREATED RATS[1]

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HYPOTHYROIDISM induces an increase in plasma globulin concentration in the rat without significantly altering plasma albumin levels. This increase in plasma globulin was observed in adult male rats made hypothyroid by feeding thiourea (1), but at the same time a decrease in seminal vesicle weight was observed thus raising the question as to the part played by androgen in these plasma protein changes. The decrease in seminal vesicle weight could not be correlated with a decrease in food intake or with total gonadotrophic hormone content of the pituitary (2). Smelser (3) previously observed a seminal vesicle weight loss in the absence of a change in pituitary gonadotrophic hormone content in thyroidectomized rats. The effect of thiourea on seminal vesicle weight suggested an androgen deficiency which, in view of the known relationship between androgens and protein metabolism (4), raised a question as to the importance of hypogonadism in plasma protein changes. The clinical studies involving androgens and serum proteins have provided varied results (5–9) and except for the studies of Abels and his co-workers (8) have involved only total serum proteins. In the experiments reported here the plasma globulin concentrations can be seen to be altered by androgen. Since the influence of androgen on organs other than those of the reproductive system is still controversial, the autopsy data is also presented.

MATERIALS AND METHODS

Male rats of the Long-Evans strain were used when 150 to 155 days of age. The rats were caged in pairs in metabolism cages for measurement of daily food intake. The diet consisted of Purina calf chow plus 10 per cent meat scrap, the meat scrap containing 55 per cent protein. The protein content of this diet was 33 per cent. All animals had been raised on the same diet used during the experimental regime and received supplements consisting of a mixture of two-thirds mazola and one-third cod liver oil on bread twice weekly and of fresh carrots once each week.

Castrated rats were injected on the day following gonad removal. Testosterone propionate (Perandren, Ciba[2]) was administered subcutaneously daily in oil (0.1 cc). After a 20- to 25-day experimental period, the rats were lightly anesthetized with ether and bled from the heart. Hematocrit, non-protein nitrogen, total plasma protein, albumin and globulin were determined by methods

reported previously (1). Each rat was autopsied and the fresh weight of the pituitary, adrenal, thyroid, seminal vesicles, kidneys, liver and, in some instances, ventral prostate and spleen were recorded.

RESULTS

Castrated rats were permitted to eat *ad libitum* and food consumption was at a normal level although the castrated rats gained on the average only 5 gm. as compared to a 17-gm. body weight gain by the controls. The castrated rats exhibited total plasma protein concentrations well above normal and due entirely to a rise in plasma globulin (table 1). Plasma albumin, non-protein nitrogen and hematocrit levels were not significantly altered.

Replacement therapy in castrated rats was initially studied at 1.25 mg. daily. Table 1 reveals that the androgen at this level will prevent the rise in plasma globulin

TABLE 1. PLASMA PROTEIN CONCENTRATIONS IN CASTRATED AND TESTOSTERONE
PROPIONATE TREATED RATS

NO. OF RATS	TREATMENT	BODY WT., START—END	HEMATOCRIT	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN
		gm.	*%*	*mg./100 cc.*	*gm./100 cc.*	*gm./100 cc.*	*gm./100 cc.*
20	Normal	235–252	45.4 ± 0.7[2]	54 ± 0.8	6.37 ± 0.07	3.86 ± 0.09	2.51 ± 0.10
20	Castrated	268–273	44.5 ± 0.7	54 ± 2.4	6.65 ± 0.10	3.69 ± 0.10	2.96 ± 0.13
8	Castrated + 0.1 mg. T.P.[1]	270–280	46.6 ± 0.9	66 ± 4.2	6.04 ± 0.13	3.60 ± 0.10	2.44 ± 0.13
10	Castrated + 0.25 mg. T.P.	240–258	46.5 ± 1.0	96 ± 5.0	6.21 ± 0.15	3.76 ± 0.14	2.45 ± 0.11
11	Castrated + 1.25 mg. T.P.	272–262	48.2 ± 0.8	58 ± 2.7	6.44 ± 0.09	3.87 ± 0.09	2.57 ± 0.13
11	Normal + 0.5 mg. T.P.	310–322	47.0 ± 0.7	54 ± 0.9	6.13 ± 0.12	3.62 ± 0.11	2.51 ± 0.15
14	Normal + 1.25 mg. T.P.	259–264	49.0 ± 0.8	61 ± 3.9	6.68 ± 0.12	3.91 ± 0.09	2.77 ± 0.10
8	Normal + 2.5 mg. T.P.	280–279	50.0 ± 0.7	68 ± 3.9	5.92 ± 0.18	3.92 ± 0.31	2.00 ± 0.17

[1] T.P. = Testosterone propionate. $^{2}\epsilon = \sqrt{\dfrac{\Sigma d^2}{N(N-1)}}.$

concentration and, in fact, maintains plasma levels at normality. These rats, however, lost on the average of 10 grams of body weight during the experimental period and on autopsy the excessively stimulated accessory sex organs indicated the hormone overdosage (table 2). Resorting to daily dosages of 0.1 mg. and 0.25 mg., it was found that both of these amounts would adequately prevent the rise in plasma globulin concentration while permitting body weight to increase. Using seminal vesicle weight as a criterion, it was apparent that 0.1 mg. of testosterone propionate more closely approximated a physiological dose than the larger amounts used (table 2). For no apparent reason the non-protein nitrogen of castrated rats receiving 0.25 mg. of the androgen was very high (table 1).

TABLE 2. ORGAN WEIGHTS OF CASTRATED AND NORMAL RATS TREATED WITH TESTOSTERONE PROPIONATE

NO. OF RATS	TREATMENT	PITUITARY	ADRENAL	THYROID	TESTIS	SEMINAL VESICLE	VENTRAL PROSTATE	SPLEEN	KIDNEY	LIVER
						mg./100 gm. body wt.				
20	None	3.0 ± 0.3^1	10.0 ± 0.2	8.0 ± 0.1	1063 ± 77	377 ± 115	80 ± 7	314 ± 29	919 ± 37	3772 ± 147
20	Castrated	3.4 ± 0.1	9.7 ± 0.4	6.8 ± 0.2		54 ± 2.0	11 ± 0.6	422 ± 33	849 ± 25	3769 ± 138
8	Castrated + 0.1 mg. T.P.	3.5 ± 0.1	10.2 ± 1.0	9.3 ± 0.2		409 ± 19	121 ± 12	609 ± 62	1006 ± 37	4762 ± 151
10	Castrated + 0.25 mg. T.P.	2.8 ± 0.1	10.0 ± 0.5	7.7 ± 0.6		636 ± 30	173 ± 10	516 ± 43	947 ± 41	4459 ± 132
11	Castrated + 1.25 mg. T.P.	2.9 ± 0.1	8.9 ± 0.5	7.3 ± 0.4		962 ± 28	218 ± 10	468 ± 57	989 ± 47	3866 ± 221
11	Normal + 0.5 mg. T.P.	2.5 ± 0.1	7.0 ± 0.5	7.1 ± 0.4	805 ± 48	739 ± 24			844 ± 42	3837 ± 295
14	Normal + 1.25 mg. T.P.	2.8 ± 0.1	8.3 ± 0.4	6.4 ± 0.3	990 ± 37	990 ± 37	250 ± 13	325 ± 15	911 ± 27	3665 ± 132
8	Normal + 2.5 mg. T.P.	2.8 ± 0.1	9.9 ± 0.5	6.8 ± 0.4	$96? \pm 41$	969 ± 41	256 ± 16	339 ± 40	984 ± 42	3634 ± 162

$$^1 \epsilon = \sqrt{\frac{\Sigma d^2}{N(N-1)}}.$$

The effect of testosterone propionate on the plasma protein concentrations of normal rats was studied following administration of daily dosages of 0.5 mg., 1.25 mg. and 2.5 mg. The smaller dosage permitted normal body weight increases but the larger doses suppressed the growth rate. The 1.25 mg. and 2.5 mg. amounts of androgen had a tendency to increase hematocrit and non-protein nitrogen whereas the effect on plasma protein concentrations was varied (table 1). Unlike the castrated rat, a daily dosage of 0.5 mg. of androgen failed to alter the plasma protein concentrations in the normal rat. The 1.25 mg. dosage was followed by a tendency for total protein levels to be increased, due to an increase in globulin, but the 2.5 mg. dosage exercised quite the opposite effect on the globulin levels. This latter dosage provided data that were widely variable.

After castration, the reproductive organs were atrophic but significant weight changes were not recorded for the pituitary, adrenals or liver. Kidney and thyroid weights had decreased somewhat whereas splenic weight was increased (table 2). Testosterone propionate at 0.1 mg. daily readily maintained the accessory reproductive organs but larger doses induced excessive stimulation. Any tendency toward pituitary hypertrophy was abolished by the androgen but a weight increase of the spleen, kidney and liver, over that of the normal as well as the castrated rat, was obtained with 0.1 mg. daily. Increased dosages were less effective so that administration of as much as 1.25 mg. daily abolished the liver hypertrophy and retained the kidney weight at normalcy. Spleens of the androgen treated castrates were heavier in each group but did not exceed castrate level when a 1.25 mg. dosage was used. In normal rats, the androgen exerted the anticipated effects on the reproductive system but, despite the protein anabolic action assigned to testosterone, it failed to increase kidney, liver or splenic weights in relationship to body weight and body weight gain was not accentuated. Thyroid weight and in some cases adrenal weight was decreased by androgen (table 2).

DISCUSSION

Castration induced a significant rise in plasma globulin in 20 days in adult rats. Since a slight but not significant fall in plasma albumin was usually obtained the resultant change in total plasma protein was frequently not striking. One might consider this plasma change as an indirect effect via the thyroid since the BMR of a castrated rat is below normal (10). However, an increase in NPN and a decrease in hematocrit as typical changes in hypothyroidism (1) did not occur after castration. The adrenal cortex may be involved (11) although in this regard adrenal hypertrophy did not occur in our animals and Smith (12) has observed increased adrenal activity only after 56 days of gonadectomy. Fasting will excite the adrenal cortex and may lower the BMR, but this does not seem capable of explaining the changes observed since as much as a 50 per cent reduction in food intake will rarely increase the plasma globulin level significantly.

Although many studies have demonstrated the nitrogen-retaining action of testosterone propionate in castrated animals these studies have not involved the plasma. In the eunuchoid, androgen administration failed to alter plasma protein levels (5), but in the castrated rat the hormone prevented the rise in plasma globulin following

castration only. Examination of total protein levels would not clearly reveal this action since the slight decrease in albumin would mask the effect. This corrective action of testosterone propionate may or may not be assigned to a thyroid-like stimulation since Kenyon (13) noted an increased BMR in androgen-treated eunuchoids but negative effects on the BMR of the castrated rat have also been reported (10, 14). Testosterone is effective in causing nitrogen retention in normal dogs and rats (15, 16) but plasma protein levels were not reported. Various clinical reports have related the plasma protein concentrations to androgen administration but the results range from a slight increase (7), to no change (9), to a decrease (6, 8). The current studies on normal rats indicate little effect with 0.5 mg. daily, a plasma protein increase with 1.25 mg. and a definite decrease due to a drop in plasma globulin concentration with 2.5 mg. These results only emphasize the need for further study and possibly why the clinical data are conflicting.

Castration has been reported to cause a decrease in liver weight with an increase to greater than normal with testosterone (17) whereas in the normal rat a decrease and in puppies an increase in liver weight followed androgen treatment (18, 19). Castration for 25 days failed to significantly alter liver weight but an increase to above normal was obtained with relatively low dosages of androgen whereas the effect was abolished with larger doses. No effect on liver weight in normal rats was recorded.

Whether or not androgens effect kidney size in the rat is as yet unanswered (4). In our studies, kidney weight/body weight was not influenced in normal rats by 0.5 to 2.5 mg. daily, but restoration of the slight kidney weight loss following castration was readily attained with testosterone propionate.

Castration led to some increase in splenic weight which was augmented by small dosages of androgen. This reaction of the spleen is unlike that of the thymus which decreases after hormone administration (20). No effect on splenic weight was obtained in normal rats with androgen when considered in relation to body weight.

Korenchevsky (21) reported a decrease in thyroid weight to body weight ratio after castration and our results suggest a similar trend. These data have been contested on the basis of actual weights and indeed our data would fail to show any thyroid effect of castration alone if actual weights were compared. Androgen administration to castrated rats keeps thyroid weight normal whereas large doses depress thyroid weight. An increase in thyroid weight in mice and an increase in mitotic figures in rats has been correlated with androgen treatment by other investigators (22, 23).

SUMMARY

Castration for 20 to 25 days increased total plasma protein concentrations in adult rats due to an increase in plasma globulin. Plasma albumin had a tendency to be reduced, but not significantly and non-protein nitrogen and hematocrit remained unchanged. Testosterone propionate prevented the plasma protein changes induced by castration but induced varied results in normal male rats.

Three weeks after castration, the reproductive system was atrophic but significant weight changes on a body weight basis were not recorded for the pituitary, ad-

renals or liver. Kidney and thyroid weights had decreased whereas splenic weight was increased. Small doses of testosterone propionate increased the weight of the spleen, liver and kidney in the castrated rat but larger doses were less effective.

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MECHANISMS OF DESOXYCORTICOSTERONE ACTION. II. RELATION OF SODIUM CHLORIDE INTAKE TO FLUID EXCHANGE, PRESSOR EFFECTS AND SURVIVAL

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THE rôle of sodium as an accessory factor in hypertension and nephrosclerosis has been emphasized during the past half century by an increasing accumulation of human and animal data (1). Inasmuch as sodium metabolism is regulated primarily by the corticosteroids, the possibility exists that the aggravation of the hypertensive states by salt results from their underlying etiologic basis in adrenal cortical dysfunction.

The hypertensive capacity of the corticosteroids is evident in the blood pressure elevations which attend cortical tumors. Its independence of sensitizing procedures as necessary adjuncts has been demonstrated (2) and confirmed experimentally (3) by investigations in which implantation of DCA pellets was followed by the development of hypertension in normal animals to which no supplementary sodium was administered.

These observations have prompted further study to determine first, if maximal increases in salt exchange could provoke hypertension in the absence of adrenocortical dysfunction and second, if the degree of hypertension induced by excessive salt-retaining hormone could be correlated with an increased level of salt intake.

EXPERIMENTAL PROCEDURES AND RESULTS

Effects of Sodium Chloride on Weight, Fluid Intake and Blood Pressure. The first experiment of this section consisted in the substitution of isotonic (0.86%) sodium chloride for drinking water in a group of 24 rats of the Sprague-Dawley strain from the time of weaning until maturity. The group was divided evenly as to sex. A control group of similar composition was maintained on water. The animals were kept in individual cages and fed the drinking fluid and Purina laboratory chow *ad libitum.* Fluid intake and weight were measured on a schedule previously described (4). The data were grouped according to 20-gm. weight intervals.

The results (fig. 1) indicated that unit fluid intake (cc/gm/day) was related inversely to weight in both groups. The intake curves for female animals closely approximated those of males in the same group, despite differences in rate of growth. These relationships in growing animals of the same species were analogous to the results obtained comparing mature animals of differing species, in which fluid exchange was found related to body weight by a fractional exponent (5).

The animals offered saline drank more than the controls throughout the weight

ranges studied.   Calculations of the ratio of average saline intake to average water
intake at corresponding weights (fig. 1), indicated that the ratio of these intakes
remained fixed despite the steady decline in unit intake which occurred as the ani-
mals matured.

In the next experiment, groups of 12 male rats were maintained on water,
isotonic, twice, and two and one-half times isotonic saline respectively for a period of
14 weeks.   The hypertonic solutions were given in order to increase not only the
overall salt exchange but the osmotic work of the kidney as well.   In addition to the
measurements previously described, the blood pressure of each animal was estimated
weekly by the tail method (6) from the eighth week onward.   All animals were
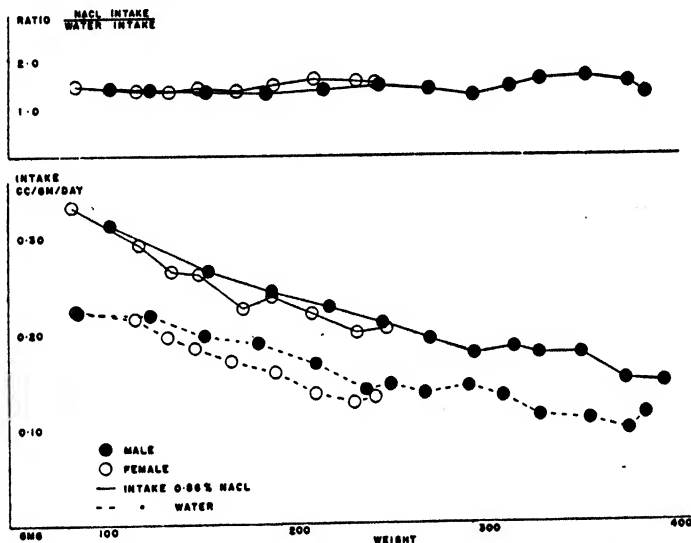
Fig. 1. RELATIONSHIP OF WATER AND ISOTONIC SALINE INTAKES
in rats as modified by growth and sex.

autopsied.   Analyses of wet and dry tissue weights were made on representative
organs of surviving animals in each group.

The growth curves of animals which drank isotonic saline did not differ signifi-
cantly from those of controls (fig. 2).   Fluid intake-weight relationships were as
previously described.

The animals given twice isotonic saline showed an initial loss of weight and an
increase in intake greater than that exhibited by the previous group.   Three of the
animals refused to drink after varying periods of time, lost further weight and died.
Survival on the contrary, as in animals forced to drink sea water (7), was characterized
by a progressive rise in fluid intake to levels three times greater than the control value,
accompanied by stabilization of weight or a small gain.   As a consequence, the
average weight of the group at the conclusion of the test period approximated its
initial value.

The animals maintained on two and one-half times isotonic saline presented an
exaggerated picture of the preceding group.   Loss of weight was progressive through-

out the greater part of the test period. As before, the animals survived if they increased their intake and died when they reduced it. Six of the group completed the entire period. Toward the end their weights had stabilized at values below the initial levels and daily fluid intake approached body weight. The animals spent most of their waking hours drinking.

Despite these marked alterations in growth, weight, water and salt exchange, none of the test groups manifested a rise in blood pressure above control levels. There were no evidences of salt or water loss by vomiting or diarrhea.

Effect of Sodium Chloride on Organ Weight and Hydration. Comparison of the percentage organ weights of the test animals with those of water-fed controls, both
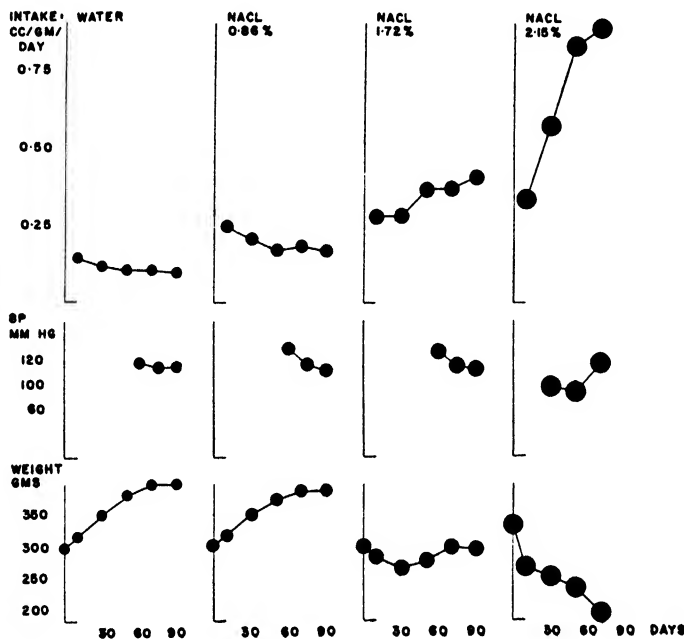
Fig. 2. FLUID INTAKE, BLOOD PRESSURE AND GROWTH of rats in relation to the salt content of the drinking fluid.

normal and starved (table 1), revealed increases in heart and kidney weight in proportion to the concentration of salt in the drinking fluid. These changes appeared too great to be explained entirely on the basis of reduced nutrition. Calculation of wet and dry weight ratios (table 2) indicated the net tissue water content of the test groups to approximate that of the controls.

The results suggest that increased velocity of fluid exchange facilitates the renal excretion of sodium, even when increased velocity is obtainable only at the expense of drinking still further quantities of a salt-containing solution. The effectiveness of the mechanism is evidenced by the maintenance of tissue hydration and prolongation of life in the animals which employed it. It is significant that a general rise in blood pressure was not invoked as a means of increasing glomerular filtration

pressure and consequent sodium clearance, even at levels of salt intake which reached 2 per cent of the body weight per day.

Effects of DCA on Intake. Four groups of 10 freshly weaned rats were used in the first experiment of this section. Males and females were represented equally in each group. Two of the four groups were controls, maintained on water and on 0.86 per cent sodium chloride solution respectively. The two test groups were im-

TABLE 1. PERCENTAGE ORGAN WEIGHTS OF RATS FED VARYING SALT CONCENTRATIONS IN DRINKING FLUID

	DRINKING FLUID				
	Water	0.86% NaCl	1.72% NaCl	2.15% NaCl	Water, no food
No. of animals...........	10	12	12	10	7
Body wt. (gm.).........	413	392	279	173	156
Organ (% wt.):					
Adrenals.............	0.015	0.016	0.018	0.040	0.038
Heart...............	0.37	0.41	0.51	0.68	0.53
Kidneys.............	0.84	0.92	1.2	1.3	1.1
Liver...............	4.2	4.3	4.9	4.8	2.4
Spleen..............	0.16	0.17	0.19	0.11	0.21
Testes..............	0.74	0.91	0.68	0.38	1.10

TABLE 2. PERCENTAGE WATER CONTENT IN TISSUES OF RATS FED VARYING SALT CONCENTRATIONS IN DRINKING FLUID

ORGAN	DRINKING FLUID			
	Water	NaCl 0.86%	NaCl 1.72%	NaCl 2.15%
Brain....................	76.7	78.5	79.1	78.5
Heart....................	74.8	78.8	81.6	82.0
Kidney..................	76.8	75.3	80.7	66.2
Liver...................	69.9	73.0	72.8	69.4
Muscle..................	75.2	74.6	77.2	75.1
Skin....................	63.8	62.8	63.3	69.5
Spleen..................	80.6	78.4	81.7	70.1
Average..............	74.0	74.5	76.6	73.0

planted with single 20-mg. DCA pellets after an observation period of one week. One of the test groups was maintained on water and the other on isotonic saline. Weight and fluid intake were measured continuously for a three-month period. The data were grouped for each 20-gm. weight interval.

Immediately following implantation, the level of intake rose in both test groups and reached a peak within 10 days (fig. 3). The test animals maintained on salt solution manifested a maximum rise over the corresponding control level about four times greater than that of the group maintained on water at the same drug dosage. Subsequently, the unit intakes declined but at rates greater than those displayed by

the controls. As a consequence, the intake curves of all four groups tended toward a common value with the passage of time.

The effect of dosage variation on intake was studied in a group of 12 rats maintained on saline. Six animals were implanted with single 20-mg. pellets and the remainder with ten 10-mg. pellets. Comparison of the intake curves with that of a control group (fig. 4) indicated the response to be qualitatively similar at both dosages. The magnitude of the initial rise in intake and the rate at which the intake curve returned toward the control value were greater at the higher dosage.

The sum of these results suggested that the effect of DCA on fluid exchange under various conditions of dosage and drinking fluid was the displacement of the intake curve upward from the control level. Calculations, therefore, were made of
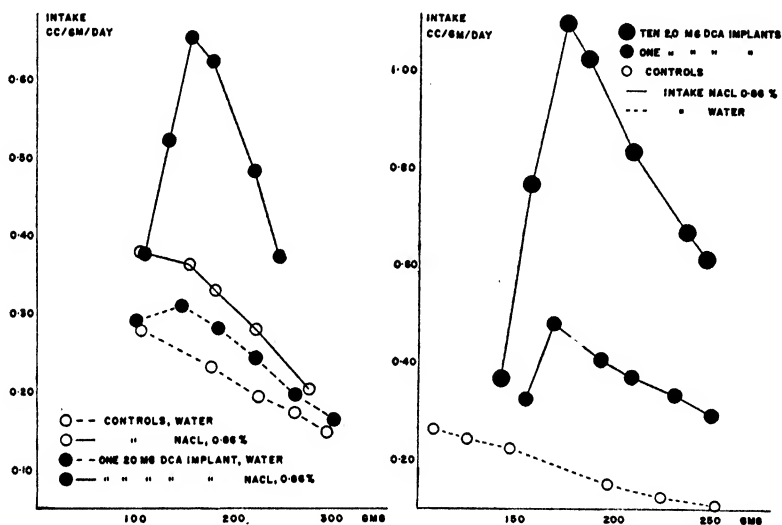
Fig. 3 (*left*). EFFECT OF DCA IMPLANTS on the intake of rats in relation to the composition of the drinking fluid.

Fig. 4 (*right*). EFFECT OF DCA IMPLANTS on the intake of rats in relation to dosage.

the ratios of the intake of each test group to the control intake at corresponding weight levels, commencing at the time of attainment of maximum increase in intake. Graphic representation of these ratios (fig. 5) indicated that the basic action of DCA under all circumstances studied was to increase the intake by a factor, the numerical value of which was fixed by the drug dosage and the composition of the drinking fluid. Once established, this ratio tended to remain more or less constant, with some overall tendency to decrease with the passage of time. The data at hand are not sufficient to determine whether this tendency represented a change in the response of the animal to the influence of a fixed dosage of drug, or a slow decrease in dosage due to gradual pellet absorption.

It would appear from these results that the secondary regression of intake levels which follows the initial DCA-induced elevation reflects mainly the magnification of a series of successively decreasing control values by a constant factor.

Further inspection of the displacements in intake curves produced under various conditions indicated that the combined effect of DCA implantation and isotonic saline administration was greater than either the sum or the product of the two separate effects. Thus, the factor of increase, which resulted from implantation of a single 20-mg. DCA pellet in animals also given saline to drink, was found equal to the product of the DCA factor alone multiplied by the square of the salt factor alone, when the numerical values of these factors were determined in groups of animals studied simultaneously. A comparison of the actual changes in unit intake which followed a combination of DCA implantation and isotonic saline administration, with the theoretical curve calculated on the above basis, showed a considerable degree of correspondence (fig. 6).
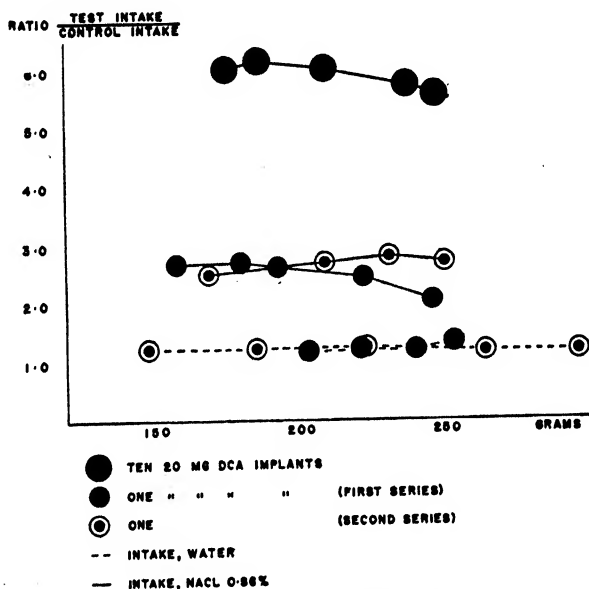
Fig. 5. RATIO OF TEST TO CONTROL INTAKE following DCA implantations as modified by drug dosage and composition of the drinking fluid.

Calculations based on a small number of data derived from animals in which ten 20-mg. pellets had been implanted indicated that the combined factor of intake increase at this higher dosage level approximated the product of the DCA factor multiplied by the cube of the salt factor.

Effects of DCA on Blood Pressure. Investigations previously reported (4) have shown that hypertension develops slowly after DCA implantation, being preceded in time by the rise in fluid intake to its peak value. It has been indicated also that salt supplementation is not required to produce blood pressure elevation (2).

These studies have been extended to a total of 113 animals. Recapitulation of the blood pressure changes (table 3) showed that the average maximum pressure of 47 control animals did not exceed 132 mm. Hg. Determinations made on 42 animals implanted with single 20-mg. DCA pellets revealed an average maximum blood

pressure of 158 mm. in those maintained on water as compared with a pressure of 176 mm. in the animals which drank isotonic saline.

In contrast to these differences displayed by water and salt-treated animals at low DCA dosages are the results in animals implanted with ten 20-mg. pellets. Twelve water-fed animals so treated averaged a maximum blood pressure of 209 mm. as compared with a value of 207 mm. in 12 similarly implanted but maintained on isotonic sodium chloride.
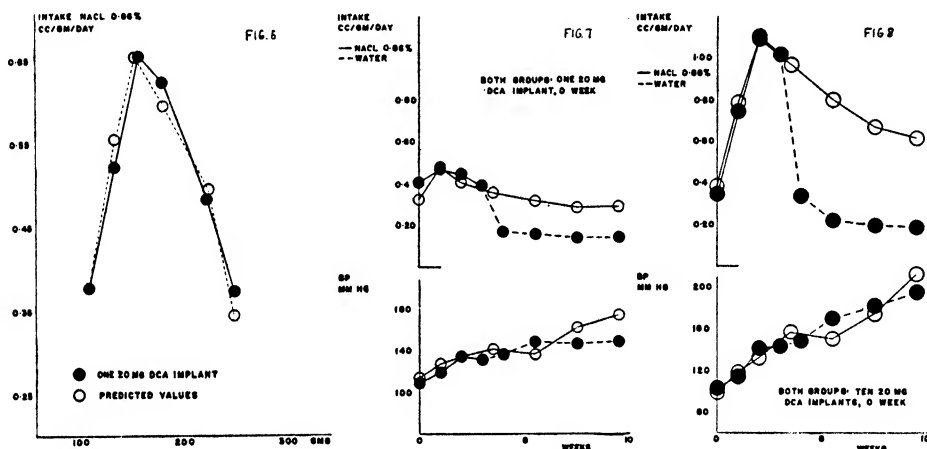
Fig. 6. COMPARISON OF OBSERVED INTAKE CURVE following DCA implantation in rats maintained on isotonic saline with the theoretical intake calculated from the changes produced by DCA and by isotonic saline administration separately.

Fig. 7. EFFECTS OF LOW DCA DOSAGES on fluid intake and blood pressure as modified by the substitution of water for isotonic saline drinking fluid.

Fig. 8. EFFECTS OF HIGH DCA DOSAGES on fluid intake and blood pressure as modified by the substitution of water for isotonic saline drinking fluid.

TABLE 3. AVERAGE MAXIMUM BLOOD PRESSURE OF RATS FOLLOWING DCA IMPLANTATION

DRINKING FLUID	CONTROLS		ONE 20-MG. DCA PELLET		TEN 20-MG. DCA PELLETS	
	No.	BP	No.	BP	No.	BP
Water	19	124	20	158	12	209
NaCl 0.86%	28	132	22	176	12	207
Average	47	128	42	167	24	208

In one experiment of this series 12 animals were implanted with single 20-mg DCA pellets and maintained on isotonic saline until the peak increase in intake had been attained, a period of approximately two weeks (fig. 7). At this time half of the animals were given water to drink while the remainder were continued on saline solution. The fluid intake of the animals placed on water immediately dropped to levels similar to those previously described for implanted animals maintained on water from the outset. However, the blood pressure of these animals continued a

slow progression to a maximum level of 149 mm. Hg, as compared with 174 mm. Hg in the animals maintained on saline throughout the test period. This experiment was repeated with 12 additional animals, in which ten 20-mg. pellets were implanted (fig. 8). The substitution of water for saline produced effects similar to those observed at the lower DCA dosage, but the blood pressure differences were not as great.

From these results it would appear that the administration of isotonic sodium chloride in place of drinking water increases the degree of hypertension developed by animals implanted with DCA, but that this potentiation is less marked when the DCA dosage is increased.

Effects of DCA on Organ Weight and Hydration. The percentage weights of representative organs from 56 DCA-treated rats were compared with those of 17 controls (table 4). The treated animals had been implanted for 10 or more weeks before spontaneous death or termination. The average body weight of test animals

TABLE 4.   PERCENTAGE ORGAN WEIGHTS OF RATS IMPLANTED WITH DCA AT TWO DOSAGE LEVELS

	CONTROLS	ONE 20-MG. DCA IMPLANT		TEN 20-MG. DCA IMPLANTS	
	Drinking fluid				
	Water	Water	NaCl 0.86%	Water	NaCl 0.86%
No. of animals...........	17	12	18	12	12
Body wt. (gm.).........	309	292	269	295	238
Organ (% wt.):					
Adrenals..............	0.021	0.019	0.024	0.025	0.028
Heart................	0.39	0.46	0.53	0.55	0.67
Kidneys..............	0.95	1.1	1.2	1.2	1.7
Liver................	4.8	4.8	4.7	5.3	5.6
Spleen...............	0.20	0.25	0.28	0.25	0.56
Testes...............	0.77	0.98	0.91	0.94	0.95

maintained on water differed little from that of controls. The earlier death of many saline-supplemented animals tended to lower the average weight in this group.

The heart and kidneys were enlarged in all test groups. Salt supplementation augmented this increase at both dosage levels. It appeared notable that the size of these two organs was as great in DCA-treated animals maintained on water as in animals which drank hypertonic saline in the absence of DCA.

The spleen and testes of the DCA-treated animals, unlike those of animals maintained solely on hypertonic saline, were perceptibly heavier than those of controls. At the higher DCA dosage level, the liver also was enlarged, a finding which may have significance in view of the rôle of the liver in DCA detoxification (8). In contrast to determinations made under other experimental conditions (9) adrenal size also tended to be increased.

Calculation of wet and dry weight ratios of representative tissues of test animals demonstrated a tendency to increased water content, more evident in the animals maintained on saline. The increase did not appear sufficient to explain the higher organ weights on the basis of edema.

Effects of DCA on Survival. A group of 39 rats was observed for 22 weeks following implantation of single 20-mg. DCA pellets. Autopsy findings showed pellet absorption to be greater than 95 per cent complete at the end of this period. Calculations of survival times (table 6) indicated that only 2 of the 19 animals maintained on water had died during this period as compared with 10 of the 22 animals given isotonic saline. Since, as previously described, the maximum pressures developed at this dosage are not much higher in the salt-supplemented animals, it would appear that the decrease in life expectancy produced by extra sodium ion in DCA-treated animals is disproportionate to its effect on blood pressure.

TABLE 5. PERCENTAGE WATER CONTENT IN TISSUES OF RATS IMPLANTED WITH DCA AT TWO DOSAGE LEVELS

	CONTROLS		ONE 20-MG. DCA IMPLANT		TEN 20-MG. DCA IMPLANTS	
	Drinking fluid					
	Water	NaCl 0.86%	Water	NaCl 0.86%	Water	NaCl 0.86%
No. of animals..............	3	3	4	4	4	3
Organ:						
Brain......................	76.7	78.5	78.8	78.7	74.0	80.9
Heart......................	74.8	78.8	78.4	80.5	78.6	81.2
Kidney.....................	76.8	75.3	75.2	78.6	77.6	79.2
Liver......................	69.9	73.0	69.5	72.8	72.1	73.1
Muscle.....................	75.2	74.6	77.4	75.0	78.9	78.1
Skin.......................	63.8	62.8	64.1	70.7	69.0	69.5
Spleen.....................	80.6	78.4	80.4	75.0	79.2	80.6
Average..................	74.0	74.5	74.8	75.9	75.6	77.5

TABLE 6. AVERAGE SURVIVAL OF RATS IMPLANTED WITH ONE 20-MG. DCA PELLET. (PERIOD OF OBSERVATION: 159 DAYS)

DRINKING FLUID	NO. OF ANIMALS	SURVIVAL *days*
Water..	17	150
NaCl 0.86%...	22	125

The survival period of the 12 animals which did not live out the 22-week period averaged 83 days. This time corresponded closely with the interval of maximum blood pressure increase as determined by serial measurements. It would seem, therefore, that DCA hypertension and its anatomical sequellae do not represent an irreversible threat to the life of the animal, but are lethal only during the period of maximum drug action. These results suggest that if human hypertension is sustained by a mechanism involving increased cortical activity, cure may follow the removal of the offending instrument or the nullification of its activity.

SUMMARY AND CONCLUSIONS

Increased sodium intake in rats up to two per cent of body weight per day was accompanied by elevation of fluid exchange, increased heart and kidney weight and

reduction in growth rate, but did not provoke hypertension. The data indicate that increased velocity of fluid exchange represents a mechanism for augmenting the renal excretion of sodium, even under circumstances in which the increase in velocity entails further intake of a salt-containing solution.

The primary effect of DCA was to elevate the level of fluid exchange over control values by a ratio fixed by the dose of the drug and the amount of supplementary salt administration. Hypertension was a subsequent development. The magnitude of DCA-induced hypertension did not correlate with the level of salt exchange, although it was augmented by salt administration, particularly at low DCA dosage levels. The effects of salt supplementation of DCA-implants on heart and kidney weight and on survival were disproportionate to the measured augmentation of blood pressure elevation. The resemblance between the action of salt in the DCA-induced hypertension of animals to that in essential hypertension lends additional support to the hypothesis that the mechanism of sustained pressure elevation in the human involves increased activity of the adrenal cortex. The favorable survival time of DCA-treated animals, once the period of most intense drug action had been passed, suggests the possibility of arresting human hypertensive disease either by removal of the adrenal cortex or by nullification of the activity of its salt-retaining steroids.

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COMPARISON OF CYCLOPROPANE AND ETHER ANESTHESIA ON LYMPH PRODUCTION

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CYCLOPROPANE anesthesia is accompanied by several undesirable effects. In some cases these are of serious nature and uncertain origin (for example, dangerous cardiac irritability). Until these things are explained it will always be of interest to uncover differences in physiological action between cyclopropane and the other common anesthetic agents.

The purpose of this report is to describe a difference in lymph production found under the two anesthetic agents, cyclopropane and ether.

METHODS

Animals. This study is based upon observations made in 11 mongrel dogs. They were allowed water but were fasted for 18 hours before the experiment. Immediately preceding the experiment half of the animals were given 20 cc/kg. of physiological saline over a period of an hour. This made no appreciable difference in the results and will not be discussed further. Blood pressure was determined by the usual direct method. Mean arterial blood pressure, pulse and respiratory rates were recorded at 20-minute intervals. They were comparable for the two agents. Rectal temperatures were observed at half-hour intervals. Two animals were eliminated because of high body temperature. When the subjects are breathing through a 'to-and-fro' soda lime canister in a closed system it is especially important to watch the body temperature of the animals, for it often tends to rise unless the soda lime canister is cooled.

Anesthesia. Cyclopropane anesthesia was induced (25% cyclopropane, 75% oxygen) through a plaster cone fitted with a rubber diaphragm. The dog's nose was inserted through this. The hair of its head had previously been wet in order to reduce the danger of electrostatic sparks. Ether anesthesia was induced by the open drop system. In the case of both agents, as soon as the surgical level of anesthesia had been attained, the trachea was cannulated and connected to a closed anesthesia system with to-and-fro breathing through a soda lime canister. Dead space was kept as near normal as possible. During maintenance of anesthesia 85 to 95 per cent oxygen was administered with each agent. Sufficient anesthetic was added from time to time to maintain the same level with both agents, indicated by a sluggish corneal reflex of anesthesia. It is of course important to have a comparable level of anesthesia in all cases.

Shift from one agent to another was easy in the case of cyclopropane to ether, since the former agent is almost entirely eliminated in a few minutes of expiration into the room (ether being inspired at the same time). The reverse shift, ether to cyclopropane, was much more difficult. In this case the anesthesia apparatus was disconnected at the tracheal cannula and the animal allowed to breathe into the air. At the same time a fine rubber catheter was inserted into the trachea, through the cannula, while the dog breathed into the room and pure cyclopropane was administered through the catheter in amounts adequate to keep the dog quietly anesthetized. After about 45 minutes the ether content of the body was greatly reduced. Although some odor of ether always persisted, the concentration was far below that required

TABLE 1. CYCLOPROPANE ANESTHESIA FOLLOWED BY ETHER

NO.	WEIGHT	SEX	CYCLOPROPANE						ETHER					
			Lymph		Blood			Resp/ min.	Lymph		Blood			Resp/ min.
			Flow	Protein	Hema-tocrit	Protein	Mean arterial pressure		Flow	Protein	Hema-tocrit	Protein	Mean arterial pressure	
	kg.		*mg./ min.*	*gm. %*	*%*	*gm. %*	*mm. Hg*		*mg./ min.*	*gm. %*	*%*	*gm. %*	*mm. Hg*	
1	13.6	♀	84	2.82	56.3	6.12	150	30	144[1]	1.98	61.4	6.77	125	39
2	11.0	♂	108	1.53	47.0	6.01	152	60	161	1.54	51.1	6.16	118	71
4[2]	12.2	♀	100	2.20	49.5	7.05	153	80						
5	12.5	♀	74	3.71	48.0	7.02	118	78	112	3.38	51.2	7.12	108	52
11	12.5	♂	88	2.35	54.2	6.94	165	59	140	2.47	59.0	7.09	140	62
12	10.5	♂	56	2.95	45.8	7.65	132	100	140	2.65	45.0	7.87	135	90
Means with S. E.			85 ±7.6	2.59 ±0.30	50.1 ±1.7	6.80 ±0.25	145 ±6.9	68 ±9.8	139 ±7.9	2.40 ±0.31	53.6 ±3.0	7.00 ±0.28	125 ±5.7	63 ±8.6

[1] One side doubled.   [2] The last half of this experiment was interrupted by a technical accident.

for anesthesia. The trachea was then reconnected to the closed anesthesia apparatus and a mixture of cyclopropane and oxygen administered as previously described.

Lymph Collection. Lymph was obtained from the cervical lymphatics by the method of McCarrell (1), in which a motor is used to nod the head at the rate of 16 times per minute. *Exact* reproducibility of the motion of the head of a given dog from one period to another is necessary for quantitative comparison of lymph flow from one collecting period to another. Both main cervical lymphatics were cannulated. In case these vessels were double on a side, often the case, one was tied off. A few particles of heparin were introduced into each cannula and the lymph was collected regularly at five-minute intervals. This was placed in weighed tubes, stoppered, the weight of the lymph determined, and the flow expressed in milligrams per minute. Averages were based upon 45-minute collection periods. Recorded collections were not made until the lymph flow had settled down to a uniform rate, after the start of the experiment or after shifting from one agent to another. This required usually 20 to 45 minutes.

The protein content of the lymph and the plasma (venous blood) were determined refractometrically.   The hematocrit was determined in venous blood.   The blood samples were drawn about 45 minutes after induction of the respective anesthesias.

RESULTS

The results are shown in tables 1 and 2.   There is wide variation in the lymph flow from one dog to another under a given agent.   The variation in lymph production from dog to dog tends to hide the ether or cyclopropane effect in the 'average' animal.   This variation is in accord with our previous experience (2).   An important point is the fact that each single experiment shows a difference in the same direction: the lymph flow under cyclopropane is always less than it is under ether regardless of which agent is administered first.   In absolute terms, the magnitude of these changes is not trivial.

TABLE 2.   ETHER ANESTHESIA FOLLOWED BY CYCLOPROPANE

			ETHER						CYCLOPROPANE					
			Lymph		Blood			RESP/ MIN.	Lymph		Blood			RESP/ MIN.
NO.	WEIGHT	SEX	Flow	Protein	Hematocrit	Protein	Mean Arterial Pressure		Flow	Protein	Hematocrit	Protein	Mean Arterial Pressure	
	kg.		*mg/min*	*gm. %*	%	*gm. %*	*mm. Hg*		*mg/min.*	*gm. %*	%	*gm. %*	*mm. Hg*	
3	12.3	♀	152	3.08	58.5	6.25	98	30	91[1]	2.45	60.6	6.89	103	50
7	12.7	♀	63	2.57	56.2	6.63	102	44	45	2.79	54.0	6.23	93	38
9	9.0	♀	111	2.24	57.0	7.63	118	68	85	2.15	61.0	7.98	130	59
10	12.5	♀	136	3.03	58.0	7.33	110	63	69	2.93	53.3	7.07	117	70
13	10.5	♂	77	2.60	56.5	7.24	102	58	62	2.45	57.5	7.70	126	59
Means with S. E.			108 ±16.9	2.70 ±0.16	57.2 ±0.4	7.02 ±0.25	106 ±3.6	53 ±6.9	70 ±8.2	2.55 ±0.44	57.3 ±1.6	7.17 ±0.31	114 ±7	55 ±5.3

[1] Collection started only 20 min. after last ether period.

The experiments shown here represent consecutive cases, with two exceptions: *Experiments 6* and *8* were not included for the reason that these dogs were found to have high temperatures, 41.1° C and 42.0° C., respectively, early in the procedure. No striking protein or hematocrit differences were observed.

DISCUSSION

Probably the correct explanation for the difference in lymph flow observed here is that the capillary filtering surface is less under cyclopropane than it is under ether.   Indeed, this must be the explanation until the unlikely possibility that ether increases the permeability of the capillary wall can be ruled out by direct observations.   However, the same protein concentration found in the lymph under the two agents supports the view that there is no great change, if any, in capillary permeability under the two agents.

Interesting support, from an entirely different approach, for the view that the

capillary circulation is less extensive under cyclopropane than it is under ether is found in the observations of Zweifach *et al.* (3). They studied the blood flow in the capillary bed of the dog's omentum during graded hemorrhage under several anesthetic agents. Their observations indicated that as bleeding continues under cyclopropane, the number of open capillaries remains normal for one-half hour (average) with constriction (flow through the most direct channels) persisting for the subsequent three hours; whereas with ether, under the same circumstances, the number of open capillaries remains normal for one and one-half hours (three times as long as with the cyclopropane). In the case of ether, the normal period is followed by a relatively brief (45-minute) constrictive phase (one-quarter as long as with cyclopropane) and then an unrestricted condition follows, with widespread flow through all capillaries, under ether but not under cyclopropane.

Anesthetic effects under circumstances where continued hemorrhage is a factor may not be comparable to conditions where this is not the case; however it seems probable that this situation is comparable to the continued stress of surgical procedure with some hemorrhage. It has been the general observation (4) of this group that, under cyclopropane anesthesia, the precapillary sphincters tend to be closed with reduction of the capillary circulation with most of the blood in the major vessels. Here a 'thoroughfare channel' or 'extended arterio-venous anastamosis' serves to transport blood from the arteriole to the venule with less utilization of the capillary bed than with ether. The head of pressure in the thoroughfare channel is greater under cyclopropane with flow more rapid than it is under ether.[1]

These findings are in accord with our observation that more lymph is produced under ether than under cyclopropane anesthesia. Presumably the tissues would be nourished less well under cyclopropane than under ether. Moreover, with the precapillary entrance tending to be cut off, under cyclopropane, and direct routes of flow from arteriole to venule being utilized, the venous flow would be unusually effective in drawing tissue fluid into the venous end of capillaries and less would go to the lymphatics. Under this circumstance, too, less oxygen and other food supply would apparently be available for the distant cells' nutrition than if the tissue fluid followed its usual washing flow from capillary to lymphatic.

Our findings, as well as those of Zweifach and his associates, are in accord with a new interpretation of the often mentioned clinical observation that the venous blood of patients under cyclopropane is unusually well oxygenated. The question can be raised: Is this because the blood proceeded so directly from arteriole to venule that oxygen could not be claimed by the tissues? Is it possible that the tissues under cyclopropane are in actuality poorly oxygenated, poorly nourished? Does this explain the collapse sometimes seen at the end of surgery when patients are taken off the oxygen-rich atmosphere of closed anesthesia?

While lymph flow itself may not necessarily be an important factor in the nutrition of most tissues during anesthesia, there is still the problem of what the effect of an agent might be that reduced lymph flow in tissues where continuous motion is present, that is, in the lungs and in the heart.

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[1] Could this explain the often-reported greater bleeding during surgery under cyclopropane than under ether?

Using still another approach and another part of the body we (5) have made observations that are consistent with the view that cyclopropane restricts the capillary circulation more than ether. Under ether in man, the average glomerular filtration rate fell 21 per cent (in comparison with the pre-anesthetic level), and 32 per cent with cyclopropane. Average effective renal plasma flow fell 39 per cent with ether and 52 per cent with cyclopropane. In the 15 men studied, these reductions were always greater under cyclopropane than they were under ether.

SUMMARY

When the effects of cyclopropane and ether on lymph production are compared under standardized conditions in dogs, cyclopropane is found to result in the production of less lymph than ether, regardless of which agent is administered first. This is interpreted as reflecting a smaller effective capillary circulation under cyclopropane than under ether. Support for this view is found in the direct observations of the dog's mesentery by Zweifach *et al.* and in the observations of Burnett *et al.* on kidney circulation in man.

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ALKALI THERAPY OF AMMONIUM CHLORIDE ACIDOSIS IN DOGS[1]

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IN SPITE of rather extensive use of alkali (sodium bicarbonate or lactate) by parenteral routes for the prompt correction of severe states of acidosis of metabolic origin, there is lack of agreement concerning its clinical value and even its need. It is generally recognized that, if renal dysfunction resulting from severe states of dehydration can be corrected by increasing the water content of the blood, the kidneys are capable in the majority of instances of effecting the necessary readjustments in the acid-base relationship. The chances of establishing and especially of maintaining such an adjustment are of course increased when the underlying disturbance, e.g. diarrhea or diabetes, is also brought under clinical control.

The speed of recovery from severe states of acidosis has been significantly increased by the prompt administration of alkali by parenteral routes. On occasion it has even seemed that recovery might have been related to the promptness of the correction of the acidosis.

A causal relationship, however, is difficult to prove in clinical practice because of the uncertainties involved in evaluating other variable factors and hence of establishing an adequate control series.

The problem is whether there exists at any time during the course of a severe degree of acidosis a stage when otherwise irreversible or irreparable damage to important cellular structures of the body could be averted by the administration of an appropriate amount of alkali. The experimental work which is described here was designed in the hope that it might be helpful in the ultimate solution of this problem.

METHOD

Mongrel dogs were used, weighing from 5 to 25 kg. The animals had access to food (hospital scraps) and water *ad libitum* at all times. Severe degrees of acidosis were induced by administration of 4 per cent ammonium chloride solution by stomach tube. The amount of NH_4Cl given was approximately 100 cc/10 kg. of body weight per dose. Administrations were made at 2-hour intervals, between 10 a.m. and 4 p.m. When emesis occurred shortly after administration, the dose was usually repeated. The total number of days of administration varied. It was continued in each dog until a low pH level (7.10 or less) existed at the end of the day (short duration acidosis), or was maintained throughout the night or longer (longer duration acidosis). Dogs in the 'longer duration acidosis' group required 2 to 10 days of administration of ammonium chloride to meet the required criterion of a morning pH below 7.10 on one or more mornings.

The ease with which an individual dog could be brought into the acidotic state depended in

part upon whether it continued to take food or not. Those animals which did so were much more resistant to the development of acidosis. All dogs lost some weight during the course of NH_4Cl administration. Partial fasting and dehydration incident to excessive polyuria were variable contributing factors to this weight loss.

When each dog reached the desired acidotic state, it was either treated with parenteral sodium bicarbonate or was kept as an untreated control. In the treated dogs, the dose of bicarbonate was estimated on the CO_2 content of serum and body weight (1, 2). When the dogs died or were terminated, the brain, spinal cord and certain peripheral nervous tissues were prepared for subsequent serial section by Dr. Jean K. Weston. Other organs were prepared for routine microscopic study. These findings will be reported later when the time-consuming examinations of serial sections are completed.

Jugular blood samples were collected, allowed to clot and serum removed under oil, attention being given to the precautions recommended by Austin *et al.* (3). The carbon dioxide content and *p*H values were routinely determined and in many cases total base and chlorides were also measured. The determinations of carbon dioxide content were made with the manometric blood gas apparatus of Van Slyke and Neill (4). The *p*H values were obtained with the Beckman glass electrode *p*H meter (model G) held at 38° C. by an air bath. A hypodermic type of cell was used. Chlorides were determined by the open Carius method (5). Total base concentration of serum was determined by the Polis and Reinhold method (6), which is based on an ion exchange reaction by a cation-adsorbing resin.

RESULTS

Despite vigorous efforts, the serum *p*H of 18 dogs never could be brought to a level of 7.10 with ammonium chloride by stomach tube, even though administration was continued for periods up to three months. However, in 90 dogs the serum *p*H was brought to a level below 7.10. This latter group forms the basis for this report.

In order to emphasize the rôle of the duration of a depressed *p*H the dogs were separated into *a*) those with a *p*H below 7.10 for 8 to 24 hours (short duration acidosis) and *b*) those with a *p*H below 7.10 for more than 24 hours (longer duration acidosis).

Dogs with pH 7.10 or Below for 8–24 Hours (Short Duration Acidosis)

1. Untreated Group. Of these 77.3 per cent (17 dogs) recovered and 22.7 per cent (5 dogs) died. For the first few days of ammonium chloride administration there was a tendency for the *p*H to return to normal values during the night after having been lowered toward 7.10 at the end of the day. The extent of the nocturnal recovery became progressively less as the period of treatment increased. The characteristic pattern of recovery is illustrated in figure 1. Dogs which retained their appetites had a better recovery response than those which did not eat well. The depression of CO_2 content was seldom below 9 mEq/l. Total base of the serum was usually depressed and serum chlorides always were elevated. The *p*H of dogs which recovered was below 7.10 on one to four occasions, those which died were below this level on only one occasion.

2. Treated Group. In this group 80 per cent (12 dogs) recovered and 20 per cent (3 dogs) died. A quantity of 5 per cent sodium bicarbonate was given by vein which was expected (1) to raise the CO_2 content to 27 mEq/l. The acid-base condition was occasionally determined one to two hours after treatment and regularly on the next morning. The *p*H and CO_2 content achieved normal values over a number of days. The percentage of dogs recovering was essentially the same in treated

and untreated groups.   In no instance was alkalosis produced.   Figure 2 shows a typical case which was treated and recovered.   Dogs which recovered had pH values of 7.10 or less one to three times; those which died had such values one or two times.

Data on all dogs with a pH of 7.10 or below for 8 to 24 hours are included in table 1, A and B.

Dogs with pH 7.10 or Below for More Than 24 Hours (Longer Duration Acidosis)

1. *Untreated Group.*   26.3 per cent (5 dogs) of this group recovered while 73.7 per cent (14 dogs) died (without therapy).   There was a tendency for a nocturnal return of the pH from low values toward normal, as described for the group of dogs,
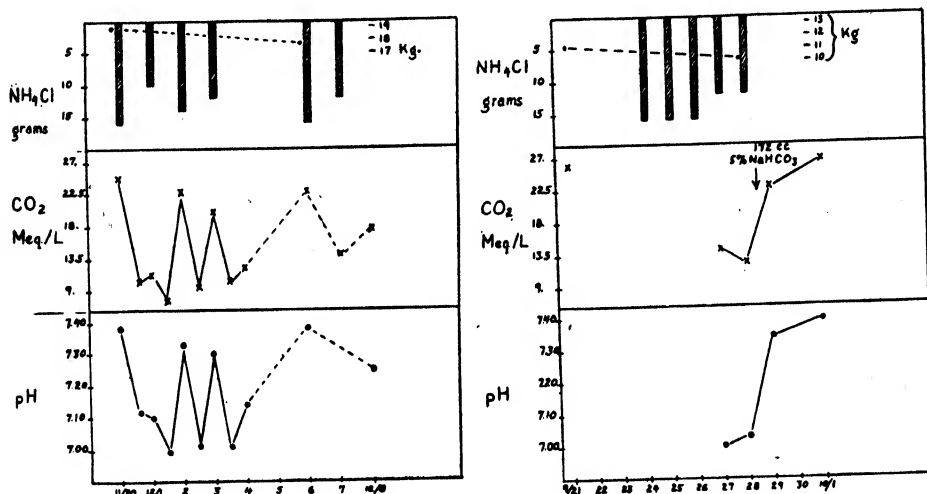
Fig. 1 (*left*).   *Dog 9.*   ACIDOSIS OF LESS THAN 24 HOURS' DURATION.   This chart shows three periods of nocturnal recovery from pH values below 7.10 at the end of the afternoons of Dec. 1-3 to values above 7.10 on the mornings of Dec. 2-4.   Dotted lines show *recovery without treatment* to Dec. 6 when another course of ammonium chloride was started.

Fig. 2 (*right*).   *Dog 751.*   ACIDOSIS OF LESS THAN 24 HOURS' DURATION.   First two pH values are P.M. samples.   This chart shows *recovery following treatment* after second sample on Sept. 28.   The last two pH samples on Sept. 29 and Oct. 1 are A.M. samples.

having a shorter duration of acidosis.   Despite the more prolonged period of acidosis depression of CO_2 content was no greater than values in the range of 9 mEq/l.   Total base of the serum was more often increased than not and serum chloride was most often increased.   Dehydration was progressive as shown by increasing hematocrit values.   Dogs of this group rarely ate much, hence weight losses were sometimes severe.   It should be noted that about three times as many untreated dogs died if the acidosis persisted more than 24 hours.   Figure 3 depicts the data from a typical case which died.   The pH values of all dogs in this group were below 7.10 two to six times.

2. *Treated Group.*   Of this group, 55 per cent (14 dogs) recovered and 45 per cent (10 dogs) died.   The intravenous bicarbonate therapy was identical with that used in the shorter duration group and pH values and CO_2 content returned more

TABLE 1A AND 1B. SHORTER DURATION ACIDOSIS

PROCEDURE	CONTROL pH	LOWEST pH	CONTROL CO_2 mEq/l.	CO_2 AT LOWEST pH	CONTROL Cl mEq/l.	Cl AT LOWEST pH	CONTROL TOTAL BASE mEq/l.	TOTAL BASE AT LOWEST pH
Untreated... Recovered...	7.378 ±0.045[1] 7.29–7.48 (17)	7.014 ±0.062 6.85–7.09 (17)	25.61 ±1.96 21.71–28.42 (17)	10.40 ±2.07 7.21–14.41 (15)	105.6 ±3.7 98.5–110.4 (15)	122.4 ±6.4 115.7–136.0 (13)	159.1 ±5.9 147–171 (14)	154.7 ±6.2 145–164 (10)
Untreated... Died...	7.362 ±0.048 7.28–7.40 (5)	6.978 ±0.202 6.62–7.10 (5)	25.00 ±1.39 23.78–27.39 (5)	12.08 ±2.64 9.46–15.73 (4)	104.2 ±3.71 100.5–109.5 (5)	118.1 111.3–124.8 (2)	165 ±4.4 160–170 (5)	154 (1)
Treated... Recovered...	7.398 ±0.038 7.30–7.45 (12)	7.037 ±0.042 6.99–7.10 (12)	24.64 ±2.28 22.16–28.56 (12)	10.66 ±2.64 7.03–13.59 (5)	103.5 ±2.44 98.5–106.7 (12)	122.7 113.2–132.2 (2)	160.6 ±6.35 146–169 (11)	159 154–164 (2)
Treated... Died...	7.326 7.27–7.36 (3)	6.863 6.83–6.91 (3)	24.53 24.28–24.77 (2)	6.98 (1)	105.4 104.6–106.2 (2)		160 158–162 (2)	

TABLE 2A AND 2B. LONGER DURATION ACIDOSIS

PROCEDURE	CONTROL pH	LOWEST pH	CONTROL CO_2 mEq/l.	CO_2 AT LOWEST pH	CONTROL Cl mEq/l.	Cl AT LOWEST pH	CONTROL TOTAL BASE mEq/l.	TOTAL BASE AT LOWEST pH
Untreated... Recovered...	7.340 ±0.061 7.27–7.43 (5)	6.942 ±0.118 6.74–7.04 (5)	23.39 ±3.88 18.65–28.02 (5)	9.26 ±2.18 7.34–12.07 (4)	105.3 ±5.55 97.5–110.7 (4)	121.3 ±4.78 117.2–127.0 (4)	163 158–166 (3)	158.5 149–168 (2)
Untreated... Died...	7.414 ±0.046 7.30–7.47 (14)	6.904 ±0.125 6.59–7.08 (14)	24.01 ±2.86 20.09–30.22 (11)	6.18 ±2.52 2.77–9.28 (6)	104.6 ±5.63 94.5–112.9 (11)	125.3 ±13.9 103.6–141.8 (7)	160.5 ±5.72 147–167 (11)	156.4 ±15.0 136–169 (5)
Treated... Recovered...	7.364 ±0.074 7.25–7.51 (14)	6.90 ±0.111 6.70–7.05 (14)	25.79 ±2.02 21.44–27.97 (13)	9.86 ±4.22 5.99–19.82 (9)	105.8 ±3.5 101.2–111.7 (13)	121.0 ±11.3 101.7–133.1 (6)	160.8 ±5.1 150–169 (13)	155.0 ±11.8 140–170 (5)
Treated... Died...	7.385 ±0.074 7.29–7.48 (10)	6.845 ±0.106 6.64–7.00 (10)	24.76 ±2.51 21.66–29.28 (8)	8.54 ±0.43 7.93–8.87 (4)	103.2 ±3.93 98.1–111.3 (8)	102.3 82.5–127.5 (3)	163.9 ±4.4 158–172 (8)	144 142–146 (2)

[1] Mean with standard deviation; range; number of dogs in parentheses.

rapidly to normal than in control untreated cases which recovered.   Chances for recovery are seen to be about twice as good with parenteral administration of sodium bicarbonate as without it when an acidosis at a *p*H of 7.10 or below is maintained for more than 24 hours.   The data of a typical case which recovered are shown in figure 4.   The *p*H level of all dogs in this group was below 7.10 two to seven times. Data on all dogs with a *p*H of 7.10 or below for more than 24 hours are included in table 2, A and B.

Because it was quite possible that solutions other than 5 per cent sodium bicarbonate might be as effective in promoting recovery of animals with long standing acidosis, a limited series of dogs (ten) was treated with physiologic saline solution and 5 per cent glucose by vein.   Since the number of cases is small the exact percentage values are not significant but the data do appear to show that dogs with both short
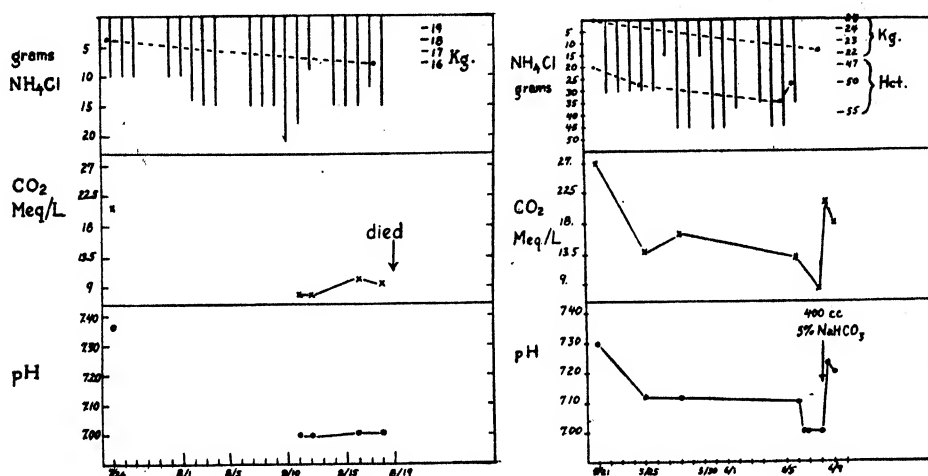
Fig. 3 (*left*).   *Dog A.*   Acidosis of more than 24 hours' duration.   This animal died without treatment.   First two *p*H values are A.M. samples, last two P.M. samples.

Fig. 4 (*right*).   *Dog 470.*   Acidosis of more than 24 hours' duration.   This animal recovered with treatment on June 8.   Last two *p*H values before therapy are A.M. samples.

and longer durations of acidosis recover equally well with glucose and saline solution, as far as ultimate survival is concerned, as with the intravenous administration of a bicarbonate solution.   Clinical improvement of these dogs was slower, however, than it was in those treated with bicarbonate.   The serum *p*H level of the bicarbonate treated dogs was elevated on the first morning following treatment from an average 6.95 to an average of 7.30 in contrast to an average elevation in the glucose and saline treated group of 7.01 to 7.23.   The data of these dogs are shown in table 3 which also summarizes tables 1 and 2.   It will be noted that the serum *p*H of the animals of this group was in the main below 7.10 a fewer number of times and that they did not have the same degree of *p*H depression as was obtained in the other two groups.

Characteristics of the Acidotic State and the Period Immediately after Treatment with Sodium Bicarbonate.   During the first few days of administration of ammonium chloride the dogs urinated frequently and profusely and drank large quantities of

water. Stools became loose, watery and were frequently stained with dark blood. Post-mortem specimens of the gastrointestinal tract from animals which succumbed showed hemorrhages of the duodenum and colon. The eyeballs were sunken, tissues were dry and there was little bleeding during the dissection. As the degree of acidosis increased there were depression of respiration, muscular weakness with ataxia, anorexia and vomiting, and finally anesthesia of superficial and deep structures. General clinical condition and the level of the *p*H could not always be correlated. Dogs with a *p*H below 7.00 did not always show the degree of clinical symptoms expected, especially during the early period of acidosis. Dogs which died had feeble heart sounds and imperceptible pulses in the agonal period. There was no evidence of disturbances in cardiac rate or rhythm up to the time of demise.

No harmful effects were noted during or after injection of 5 per cent sodium bicarbonate in any case. Loud borborygmi of the intestine were a constant finding during the injection. Dogs which had been comatose seemed to waken and become alert, and were soon walking about after the intravenous therapy with sodium bicarbonate was terminated. The rate of disappearance of symptoms was much less striking with 5 per cent glucose and 0.9 per cent NaCl.

TABLE 3

DURATION OF ACIDOSIS	% RECOVERED UNTREATED	% RECOVERED 5% BICARB.	% RECOVERED GLUCOSE AND SALINE	% DIED UNTREATED	% DIED 5% BICARB.	% DIED GLUCOSE AND SALINE
Less than 24 hours...	77.3 (17)[1]	80.0 (12)	75.0 (3)	22.? (5)	20.0 (3)	25.0 (1)
More than 24 hours..	26.3 (5)	55.0 (14)	66.6 (4)	73.7 (14)	45.0 (10)	33.4 (2)

[1] No. of cases.

DISCUSSION

In ammonium chloride acidosis of 8 to 24 hours duration, at *p*H 7.10 or below, there was little or no difference in the percentage of recoveries under the conditions of these experiments in the groups of dogs which received parenteral 5 per cent sodium bicarbonate solution, 5 per cent glucose and saline solution, or no therapy other than water and food *ad libitum*, respectively. However, when the duration of the acidosis is longer than 24 hours, the administration of 5 per cent sodium bicarbonate solution doubles the chance for recovery. Since in a small series 5 per cent glucose and saline produced just as great a percentage of recoveries as bicarbonate solution, it may be that fluid volume is more important than acid-base reaction. Three times as many untreated dogs died when the acidosis persisted more than 24 hours than when it was less than one day's duration. Apparently irreversible changes occur with much greater frequency after the first 24 hours. These changes might be secondary to loss of intracellular sodium (7) or because of intracellular dehydration, which has been considered to be the cause of death when extracellular fluids are hypertonic (8) as they were in these animals. Similar damage could depend on excessive loss of potassium (intracellular) in the diarrhea uniformly present (9). Dehydration also causes loss of intracellular potassium (10–14) and infusion of ammonium chloride causes extra excretion of potassium from cells (15).

Sunken eyeballs, dry tissues and high hematocrit are all evidences of dehydration. Oral feedings, important to recovery, contain potassium (2, 16, 17) and the dogs which ate their food well resisted both the onset of acidosis and also made a quicker recovery either with or without therapy.

Weakness of skeletal muscle may represent either the effect of an acid pH depressing the motor cortex (18) or a loss of cellular potassium from muscles incident to severe acidosis and dehydration (19) following the diarrhea (9, 19). In the first instance the paralysis would be central, in the second peripheral. Depression of the sensory cortex by an acid pH could account for the anesthesia. It was possible to painlessly expose a superficial vein without local anesthesia in the severely acidotic dogs. The lethal pH level for the intact heart *in situ* is known to be about 6.00 (20). Cardiac arrest occurs suddenly without previous abnormalities in rate, rhythm or conduction at this level of acidosis. Our lowest pH value was above this critical level and no disturbances of cardiac rate or rhythm were noted. All the dogs showed respiratory depression and not stimulation as occurs in diabetic acidosis. It is possible that the dehydration of the cells of the respiratory center caused the depression since we found extracellular fluids to be hypertonic. This is in agreement with the findings of Winkler *et al.* (8). Furthermore, patients in shock with acidosis do not show hyperpnea (16, 21). This concept is supported by the fact that CO_2 contents were usually above 9 mEq/l. and never below 4.5 mEq/l. as they often are in clinical acidosis. It may be that a respiratory depression with resultant respiratory acidosis is superimposed on the preliminary metabolic acidosis with the result that the CO_2 content is not depressed to the extent which it otherwise would be. The absence of hyperpnea with a low pH as a result of ammonium chloride acidosis is similar to that seen in man (10) although different from that reported by Haldane (22). Krogh (23) believes that the respiratory center responds to CO_2 as such and not to the hydrogen ion.

The constant presence of borborygmi during the administration of sodium bicarbonate solution may be accounted for in several ways: increased excitability, decreased threshold for nervous stimuli, and increased spontaneous rhythm of the motor cortex (and hence its autonomic representation) when the reaction is made more alkaline (18, 24, 25), direct stimulation of smooth muscle by alkali (26), or exodus of potassium from cells during alkali therapy (7, 19) with resultant stimulation (27) of smooth muscle. Bloody stools probably were the result of the hemorrhagic duodenitis and colitis which Dr. Weston demonstrated at autopsy. The findings in the intestinal tract are similar to those seen in hemorrhagic shock (28).

Acidosis does not involve changes in extracellular fluids alone (19). Our calculations to determine the amount of sodium bicarbonate to be given were based on the assumption that the bicarbonate content of extra and intracellular fluids is equal (1, 2, 17, 29) and made no allowance for depletion of intracellular sodium. This may explain failure of sodium bicarbonate or chloride solutions to fully correct the acidosis in some of the dogs after time for equilibrium was allowed. Moreover, the effect of bicarbonate varies with different patients (1, 17). One type of acidosis resulting from feeding protein milk to premature infants is characterized by almost complete loss of intracellular sodium (17). Intracellular sodium equals about one

tenth of sodium in extracellular fluids, or over one half of extracellular sodium as bicarbonate normally present (17). To fully correct an acid-base disturbance when there is a loss of intracellular as well as extracellular sodium would involve allowance for losses of sodium from the former as well as from the latter compartment. This allowance was not made.

Although 0.9 per cent sodium chloride solution alone is considered inappropiate for the initial treatment of acidosis (17), when used with glucose it increases blood flow and renal function to permit excretion of excess chloride (30, 31). This could explain the effectiveness of the glucose and saline solution in our experiments. In controlled hemorrhagic shock, sodium bicarbonate infusion given during the hemorrhage not only prevents the onset of acidosis but also the development of irreversible shock (28). It has been emphasized that hemorrhagic shock should be considered from the standpoint of an acidosis (32). It has also been shown that sodium bicarbonate protects the kidney against damage from intravenously administered half normal hydrochloric acid (33).

SUMMARY

In ammonium chloride acidosis of less than 24 hours' duration as many untreated dogs recover as do dogs treated with 5 per cent sodium bicarbonate or 5 per cent glucose in normal saline. Under conditions of these experiments treatment does not influence the course. In ammonium chloride acidosis of more than 24 hours duration treatment with either 5 per cent sodium bicarbonate or 5 per cent glucose in normal saline doubled the frequency of sur⸱ival under the conditions of these experiments. Three times as many untreated dogs died when the duration of acidosis was longer than 24 hours. Dogs treated with sodium bicarbonate made a more rapid clinical improvement from severe states of acidosis than did those treated with glucose and saline.

These data indicate that the administration of sodium bicarbonate under the conditions of these experiments is not dangerous but rather is beneficial in relation to speed of recovery.

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EFFECT OF HIGH PROTEIN AND HIGH CARBOHYDRATE DIETS ON THE ARGINASE AND PHOSPHATASES OF THE LIVER AND KIDNEY OF THE NORMAL AND ADRENAL-ECTOMIZED RAT[1,2]

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ADRENAL cortical extract administered in eight hourly doses will produce a marked increase in the 'alkaline' phosphatase of the fasted adrenalectomized (1, 2) and normal (3) rat. This effect is due to the S-hormones of the extract (3). These steroids, however, will not restore the decreased arginase activity of the liver in the fasted adrenalectomized rat (4) or influence the level in the normal rat (2) in spite of their known (4, 5) and demonstrated (2, 3) glyconeogenic effect. If, on the other hand, the S-hormones or 3 mg/day of desoxycorticosterone acetate are administered for several days, then the reduced liver arginase of the adrenalectomized rat is restored towards normal (6, 7).

It has also been demonstrated that many androgens (8) will not affect either of the above enzymes of the liver in spite of their protein anabolic effect (9) but will increase these enzymes of the kidneys of normal, adrenalectomized (2, 4) castrated and hypophysectomized rats (unpublished data). There are, however, definite species differences in the degree of these responses (10).

Since the 'S' hormones and 'N' hormones are concerned with protein catabolism and anabolism respectively, it seemed that dietary experiments might provide information concerning the significance of the above-mentioned enzyme changes.

PROCEDURE

Two types of experiments were conducted: *a*) acute, in which the rats were fasted for 24 hours and either allowed to eat *ad libitum* for 9.5 to 10.5 hours or fed by stomach tube at one- or two-hour intervals for eight hours in order to simulate the conditions of the adrenal cortical studies; *b*) chronic, in which the animals were fed the respective diets[3] (table 1) for seven days. A marked excess of thiamine hydrochloride was added to the high carbohydrate diet and of riboflavin, niacinanide and pyridoxine to the high protein diet. It is recognized now that these vitamins are essential for the proper metabolism of these foodstuffs.

The normal rats of the acute experiments and the adrenalectomized-castrated

rats of the chronic experiments were of the Wistar strain from our colony. The normal rats of the chronic experiments were of the Sprague-Dawley-Holtzman strain. The rats were kept in individual metal cages in an air-conditioned room maintained at 78 to 80°F. In the chronic experiments the normal rats were given 10 gm/day of the standard diet for seven days preceding the experimental period. The adrenal-ectomized-castrated rats had been castrated for four to six months. They were adrenalectomized two months after castration and were maintained at constant body weight by the subcutaneous implantation of a 14 to 15 mg. pellet of desoxycortico-sterone acetate[4] and 9.0 to 9.5 gm/day of the standard diet.

At the end of the experiments, the rats were anesthetized with 0.25 to 0.35 ml. of dial-urethane[4], the livers were removed and weighed on a Roller-Smith torsion

TABLE 1.　COMPOSITION OF DIETS

	STANDARD	HIGH CARBOHYDRATE (NO PROTEIN)	HIGH PROTEIN
	gm.	*gm.*	*gm.*
Casein..................................	16.7		80
Yeast...................................	9.2		10
Starch..................................		40	
Sucrose.................................	61.2	40	
Wesson oil..............................	7.4[1]	2	2
Celluflour..............................	1.8	2	2
Cod liver oil...........................	2	2	2
Wesson's salts.........................:	3.7	4	4
Thiamine HCl[2].........................		.004	.001
Riboflavin..............................		.002	.008
Niacinamide.............................		.010	.020
Pyridoxine..............................		.001	.004
Ca pantothenate........................		.010	.010
Inositol................................		.005	.005
Tocopherols[3] (34% concentrate).........	.030	.030	.030

[1] Hydrogenated vegetable oil.　[2] Crystalline B vitamins provided by Merck & Co.　[3] Provided by Distillation Products Inc.

balance. The left segment of the median lobe was saved for the enzyme determinations (2, 4) and the remainder of the liver was digested in 5 ml. of hot 30 per cent potassium hydroxide (11). The glycogen was precipitated from the digestion mixture by the addition of 20 ml. of 95 per cent ethyl alcohol, the precipitate was hydrolysed in a boiling water bath with 15 ml. of N/1 hydrochloric acid, neutralized to phenolphthalein (12) with 2 N sodium hydroxide and made to volume. Aliquots were analysed for reducing substances by the modified Somogyi method (13) and the results expressed as glucose.

The nitrogen of the urine and of the tissue enzyme homogenates was determined by the micro-Kjeldahl procedure and the urea and ammonia by aeration into 2 per cent boric acid and titration with 0.015 N hydrochloric acid.

[4] The desoxycorticosterone acetate and the dial-urethane were provided by Ciba Pharmaceutical Products Inc.

RESULTS

Acute Experiments. The arginase of the liver (table 2) was not significantly changed by any of the diets in spite of the marked differences in urinary nitrogen and urea excretion. The decreases in units per gram of tissue are due to the increases in

TABLE 2. EFFECT OF DIET ON THE URINARY NITROGEN AND THE ARGINASE ACTIVITY OF THE LIVER OF THE FASTED (24 HRS.) ADULT MALE RAT

DIET	NO. OF RATS	BODY WT.	FOOD INTAKE	URINE NITROGEN		LIVER		
				Total	Urea-N + NH_2—N	Wt.	Arginase	
							(Total U)	(U/gm.)[2]
		gm.	gm.	gm.	mg.	mg.	%	%
Fasted............	6	172		51.6		5.762	(60482)	(10450)
							(58600–64700)	(9,900–10,800)
Carbohydrate.....	6	184	10.2	47.6		8.548	−9	−41
Carbohydrate.....	6	192	7.5	30.5	22.2	7.160	−23	−38
Protein..........	6	197	7.9	224.0	181.5	6.983	+8	−15
Standard........	6	202	10.2	62.7	38.8	8.079	0	−29

[1] After a 24-hr. fast, each rat was given 20 gm. of the prescribed diet from which he ate *ad libitum* for 9.5 to 10.5 hr. before autopsy.   [2] Average control values with ranges are given in parentheses.

TABLE 3. EFFECT OF DIET ON THE LIVER GLYCOGEN AND PHOSPHATASES OF FASTED (24 HRS.) ADULT MALE RATS

DIET[1]	LIVER GLYCOGEN	'ALKALINE'		'ACID'	
		(Total U)[2]	(U/gm.)[2]	(Total U)[2]	(U/gm.)[2]
	%	%	%	%	%
Fasted..............	0.432	(10.0)[3]	(1.7)	(146)	(25.5)
		(7.7–13.6)	(1.3–2.4)	(135–150)	(23.5–28.7)
High carbohydrate (no protein)............	4.212	+40[3]	−7	+9	−27
High carbohydrate (no protein)............	0.441	−16	−33	−3	−22
High protein.........	1.033	+38	+14	+11	−12
Standard.............	1.177	+43	0	+16	−17

[1] See tables 1 and 2 for details of experimental conditions.   [2] Av. control values with ranges are given in parentheses.   [3] On activation with $MgSO_4$ the difference was only +17%.

size of the liver with the deposition of glycogen (table 3). These differences disappear when a comparison of the total enzyme activities is made.

The phosphatases of the liver show indefinite changes. The high carbohydrate diet produced an increase in the first and a decrease in the second experiment. The increase, however, probably is not real for it was reduced to 17 per cent when the levels of the $MgSO_4$ activated (14) enzymes were compared. The increases obtained with the high protein and the standard diet are only suggestive.

There were no significant changes in kidney weight or enzymes. The greatest change in any of these factors was less than 5 per cent.

The administration of 30 per cent glucose by stomach tube to 24-hour fasted normal rats at one- or two-hour intervals for eight hours increased the liver glycogen, but did not significantly affect the enzymes of the liver or kidney.   Data are not shown.

TABLE 4.   EFFECT OF DIETS (10 GM/DAY FOR 7 DAYS) ON KIDNEYS AND LIVER OF MALE RAT

Kidneys

DIET	NO. OF RATS	BODY WEIGHT		WT.	NITROGEN		ARGINASE		'ALKALINE'[1] PHOSPHATASE	
		Initial	Change				(Tot. U)[2]	(U/ gm.)[2]	(Tot. U)[2]	(U/ gm.)[2]
		gm.	*gm.*	*gm.*	*mg.*	%	%	%	%	%
Standard............	5	249	+1	1.957	29.3	3.05	(226)	(116)	(277)	(142)
High protein..........	5	249	+1	2.200	34.0	3.06	+22	+15	+25	+11
High carbohydrate (no protein)............	5	250	−13	1.611	24.7	3.09	−3	+17	−4	+11

Liver

	WT.	GLYCOGEN		NITROGEN		ARGINASE		'ALKALINE'[1] PHOSPHATASE	
		Total	%	Total	%	(Tot. U)[2]	(U/gm.)[2]	(Tot. U)[2]	(U/gm.)[2]
	gm.	*mg.*		*mg.*		%	%		
Standard..........	8.580	363	4.21	309	3.61	(86900)	(10,200)	(20.7)	(2.4)
High protein.......	9.230	224	2.41	344	3.81	+33	+23	+61	+49
High carbohydrate (no protein)......	7.610	440	5.79	227	2.97	−47	−41	+58	+77

[1] No changes in 'acid' phosphatases of kidney or liver.   [2] Av. control values with ranges are given in parentheses.

TABLE 5.   EFFECT OF HIGH PROTEIN DIET ON THE WEIGHT AND ENZYMES OF THE KIDNEY AND LIVER OF THE ADRENALECTOMIZED-CASTRATED RAT MAINTAINED WITH DESOXYCORTICOSTERONE ACETATE[1]

DIET	NO.	BODY WT.	KIDNEY			LIVER		
			Wt.	Arginase (U/gm.)	'Alkaline'[2] Phosphatase (U/gm.)[3]	Wt.	Arginase (U/gm.)[3]	'Alkaline'[2] Phosphatase (U/gm.)[3]
		gm.	*gm.*	%	%	*gm.*	%	%
Standard.......	3	280	1.839	(87) (78–95)	(120) (97–136)	8263	(4,300) (3,880–4,520)	(3.1) (2.8–3.5)
High protein....	3	274	2.173	−7	+12	9034	+18	−3

[1] Similar results obtained with incompletely adrenalectomized rats.   [2] No effect on 'acid' phosphatases of kidney and liver.   [3] Av. control values with ranges given in parentheses.

Chronic Experiments in Normal Rats.   The normal rats on the high carbohydrate diet lost considerable body and kidney weight but those given the high protein diet maintained their body and increased their kidney weight.   The nitrogen (protein) content of the kidneys changed in proportion to their weight changes (table 4).   Arginase and 'alkaline' phosphatase levels varied with the kidney weight changes.

The glycogen content and weight of the liver did not parallel each other.   The liver of the high protein fed rats was heavier but contained less glycogen than that of

the rats fed the standard diet, while the rats fed the high carbohydrate diet had smaller livers and a greater amount of glycogen.

The nitrogen (protein) content of the liver of the rats on the high protein diet was greater and that of the rats on the high carbohydrate diet was less than that of the rats fed the standard diet.

Chronic Experiments in Adrenalectomized-Castrated Rats. The high protein diet increased the weight of both the kidney and the liver of the adrenalectomized-castrated rats, but did not produce any significant changes in the enzymes of these organs (table 5). Similar results were obtained with identically treated but incompletely adrenalectomized rats.

DISCUSSION

The results of this study indicate that glyconeogenesis from exogenous protein or carbohydrate either in 'short' 10-hour, or 'long' 7-day, experiments is not accompanied by an increase in the 'alkaline' phosphatase of the liver of rats comparable to that observed during glyconeogenesis from presumably endogenous protein under the stimulus of the S-hormones of the adrenal cortex (1–3.)

The failure of the high protein diet to produce a noteworthy increase in liver arginase is in agreement with the observations of Takehara (15), Kageura *et al.* (16), Lightbody and Kleinman (17), Folley and Greenbaum (7) and Miller[5]. Lightbody and Kleinman (17), however, report that significant increases in this enzyme are obtained if male or female rats are fed a 75 per cent protein diet for three to four weeks. They attribute this to an adaptative phenomen.

It is particularly noteworthy that the high protein diet did not alter the concentration of the greatly reduced arginase activity of the liver of the adrenalectomized rat. This suggests that the loss in liver arginase activity after adrenalectomy (2, 6, 7) and also probably after hypophysectomy (18, unpublished) is not concerned with urea formation (cf. 3).

The high protein diet produces an increase in kidney and liver weight and protein as expected (19, 20) and a proportionate increase in the enzymes of the adrenalectomized as well as the normal rats. On the other hand, androgens produce a much greater increase in the enzymes, especially arginase, than in kidney weight but no change in the enzymes of the liver.

The decrease in liver arginase after seven days on the high carbohydrate-no protein diet is comparable to that observed by Lightbody and Kleinman (17) in rats fed a 6 per cent protein diet. The 'alkaline' phosphatase, on the other hand, is actually increased and the 'acid' phosphatase is spared in agreement with histochemical studies (21). Thus, the protein starved rat in calling upon its protein reserves (cf. 22, 23) decreases its requirement for arginase but increases it for 'alkaline' phosphatase. The enzymes of the kidneys of these animals were not altered in spite of the decrease in weight and protein content.

There seems to be no general correlation of enzymes with protein content of the liver or kidney. The amount of an enzyme apparently is determined by the specific metabolic demands placed upon the organ (cf. 20, 21).

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[5] Personal communication.

SUMMARY

The feeding of either a high carbohydrate (89%)-no protein, a high protein (casein 80%, yeast 10%) or a 'standard' prepared diet to 24-hour fasted adult male rats for 10 hours caused a deposition of liver glycogen and the expected changes in urinary nitrogen and urea excretion but did not change the activities of the arginase, 'alkaline' (pH 9.8) or 'acid' (pH 5.4) phosphatases of the liver or kidney. The feeding of 30 per cent glucose by stomach tube at one- or two-hour intervals also increased the liver glycogen without any changes in the activities of the liver or kidney enzymes.

The feeding of the above diets for seven days at 10 gm/day to normal 250-gram male rats showed that the high carbohydrate diet caused a loss in body and kidney weight but no change in enzyme activities. The livers of these animals lost weight and protein, but contained a large amount of glycogen. There was a decrease in arginase but a moderate increase in 'alkaline' phosphatase. The high protein diet, on the other hand, maintained the body weight and increased the kidney weight and protein with a concomitant increase in the enzymes. The liver weight, protein and enzymes were somewhat increased. The glycogen content, however, was only one-half that present in the livers of the rats fed the high carbohydrate diet.

The feeding of the high protein diets as above to completely and partially adrenalectomized-castrated rats increased the kidney and liver weights but did not change enzyme activities of these organs. Organ and enzyme changes are not comparable to those after administration of protein anabolic or catabolic steroid hormones.

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CHOLINESTERASE LEVELS IN PLASMA AND TISSUES[1]

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DURING the past 20 years frequent attempts have been made to correlate the level of cholinesterase in blood plasma with various pathological conditions, especially with those associated with a disturbed function of the nervous system and of voluntary muscles (1–10). The results of these investigations were often contradictory and on the whole disappointing. Despite the mass of data obtained no conclusions whatsoever could be drawn regarding a relationship between the cholinesterase level of blood plasma and various syndromes. However, at the time when these investigations were carried out, it was not known that there exist, in the animal body, two enzymes capable of hydrolyzing acetylcholine, true cholinesterase and pseudo-cholinesterase (11) and that only the former is essential for the hydrolysis of acetylcholine *in vivo* (12). Moreover, no method was available for distinguishing between these two enzymes and for measuring their activities separately, in sera which, in most species, contain both true cholinesterase and pseudo-cholinesterase in varying proportions. Rabbit serum, for example, contains mainly true cholinesterase, whereas pseudo-cholinesterase predominates in the serum of man (13). Furthermore, in the measurement of the cholinesterase activity of human plasma, it has been customary to use high concentrations of acetylcholine at which the activity of true cholinesterase is depressed while that of the pseudo-cholinesterase is favoured. Therefore, the results of these measurements, though reflecting the level of pseudo-cholinesterase, could give no hint of changes in the level of true cholinesterase, unless changes in the activities of both enzymes follow a parallel course, an assumption for which there has been no experimental evidence.

The present investigation was undertaken to determine 1) whether changes in the activity of the plasma pseudo-cholinesterase entail similar changes in the activity of the plasma true cholinesterase and 2) whether a correlation exists between the level of true cholinesterase in the plasma and that contained in the tissues.

METHOD AND MATERIALS

The method used for the measurement of cholinesterase (ChE) activity was that of Mendel, Mundell and Rudney (14), who showed that acetyl-β-methylcholine is hydrolyzed by true cholinesterase but not by pseudo-cholinesterase and that, conversely, benzoylcholine is hydrolyzed by pseudo-cholinesterase but not by true cholinesterase. With these two substrates it is possible, therefore, to measure the

activities of true cholinesterase and pseudo-cholinesterase separately in tissues and body fluids containing a mixture of both enzymes.

Adult, male Wistar rats weighing between 200 to 400 grams were used as experimental animals. They were killed by exsanguination through the jugular vein and the required tissues were removed. The brain was sectioned just below the level of the fourth ventricle so that approximately the same portion of brain was obtained in every case. After removing the blood vessels as completely as possible, a homogeneous suspension was prepared by grinding the tissue with three times its wet weight of distilled water.

Preliminary experiments done on three muscles, sternohyoideus, sternomastoideus and diaphragm, indicated that the former was most suitable as test object. This muscle is easily removed in its entirety and can be readily ground. In addition, of the three muscles, the sternohyoideus showed greatest activity towards acetyl-β-methylcholine. No activity was observed towards benzoylcholine, indicating that no pseudo-cholinesterase is present. A 1:3 suspension was used in the experiments to be reported.

One ml. of plasma, one ml. of muscle suspension and 0.25 ml. of brain suspension were tested for their cholinesterase activities by Warburg's manometric method at 37.5°C. in a 0.025 M solution of bicarbonate saturated with 5 per cent CO_2 in N_2 (pH 7.4). The final concentrations of acetyl-β-methylcholine chloride (Mch)[2] and benzoylcholine chloride (Bch) used in all experiments were 0.6 per cent and 0.15 per cent respectively. The total volume of fluid in the vessel was 5 ml.

Expression of Results:

a) Activity: μl CO_2 evolved by one ml. plasma in 20 minutes.

b) Q value:
$$\frac{\mu l\ CO_2}{mg.\ (dry\ weight) \times hr.}$$

EXPERIMENTAL

Relationship between the Levels of True Cholinesterase and
Pseudo-cholinesterase in Plasma

Before attempting to determine whether or not changes in the activities of the two cholinesterases in plasma follow a parallel course, conditions had to be created whereby the overall activity of the plasma towards acetylcholine would be enhanced or reduced. In preliminary experiments, it was found that thyroidectomy brought about a significant rise in the cholinesterase activity in the plasma of rats (15), whereas starvation effected a considerable decline in the activity. This latter finding is in accord with the observations of McCance, Widdowson and Hutchinson (16), who studied the effects of starvation in man.

a) *Thyroidectomy.* Sixteen male rats were thyroidectomized as outlined by Griffith and Farris (17). They were sacrificed 14 days after operation. The true cholinesterase and pseudo-cholinesterase activities of their plasma appear in section 2 of the table. From a comparison of these results with those outlined in section 1,

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[2] Merck's mecholyl.

it can be seen that an elevation of over 200 per cent in the plasma pseudo-cholinesterase activity was effected. The plasma true cholinesterase level, however, did not deviate from the normal. Thus an elevation in the pseudo-cholinesterase level of plasma is not necessarily associated with a corresponding rise in the level of plasma true cholinesterase.

b) Inanition. Ten male rats varying in weight from 300 to 400 grams were fasted for a period of 12 days. Water was supplied *ad libitum.* The weight loss ranged from 15 per cent to 28 per cent, with an average loss of 19 per cent. Both true cholinesterase and pseudo-cholinesterase activities of the plasma were tested. A comparison of the results outlined in section 3 with those appearing in section 1 of the table reveals a significant depression in the level of plasma pseudo-cholinesterase, but no change in the level of plasma true cholinesterase. Thus it is evident

TABLE 1. CHOLINESTERASE ACTIVITIES OF PLASMA AND TISSUES

SECTION	ANIMALS	PLASMA				BRAIN		STERNOHYOIDEUS MUSCLE	
		True ChE		Pseudo-ChE					
		No. of animals	Activity toward Mch	No. of animals	Activity toward Bch	No. of animals	Q_{Mch}	No. of animals	Q_{Bch}
1	Normal	28	63.0 ± 2.1[1]	27	41.0 ± 2.3	23	22.7 ± 0.7	28	5.64 ± 0.25
2	Thyroidectomized	14	61.3 ± 2.3 $t = 0.31$[2] $P = 0.76$[2]	16	97.1 ± 8.7 $t = 7.7$ $P = 0.38 \times 10^{-12}$				
3	Starved	10	64.7 ± 3.3 $t = 0.26$ $P = 0.8$	10	21.3 ± 2.7 $t = 4.8$ $P = 2.1 \times 10^{-6}$				
4	Tumor-bearing	22	25.9 ± 3.2 $t = 7.35$ $P = 0.96 \times 10^{-12}$			10	23.8 ± 0.5 $t = 0.98$ $P = 0.33$	12	5.46 ± 0.29 $t = 0.16$ $P = 0.87$

[1] $\pm$ represents the standard deviation of the mean.
[2] 't' and 'P' values are all in relation to values in normal animals.

that a depression in the pseudo-cholinesterase level of plasma is also not necessarily accompanied by a corresponding depression in the level of true cholinesterase.

Relationship between the Levels of True Cholinesterase in Plasma and in Tissues

There has been almost universal agreement that debilitating conditions are associated with a reduced ability of the plasma to hydrolyze acetylcholine. Vahlquist (18), Scoz and Cattaneo (19) and Jones and Stadie (20) report very low values in advanced tuberculosis. The latter workers also report depressed activity in advanced cancer. Milhorat (21) in his study of 109 patients chosen at random found that in cases of debilitation there was a depression which varied in a manner parallel with the clinical state. These results were confirmed by Faber (9) who investigated a group of 400 patients.

Many attempts were made in the present investigation to alter the level of true cholinesterase in the plasma of rats. Only in malignancy, however, was a significant deviation in the level of plasma true cholinesterase observed. Therefore, rats bearing a transplanted sarcoma (S39) which ranged in weight from 7 to 50 per cent of

total body weight were used to determine whether a correlation exists between the true cholinesterase levels in plasma and in tissues.   The true cholinesterase activities of the plasma, brain and sternohyoideus muscle of these animals appear in section 4. A comparison of these figures with those of section 1 of the table reveals that even though the level of true cholinesterase in the plasma is reduced about 60 per cent, the activity of this enzyme in the tissues remains within the normal range.   It would seem, therefore, that a depression in the level of true cholinesterase in the plasma is not necessarily indicative of a decrease in the level of this enzyme in the tissues.

SUMMARY AND CONCLUSIONS

Changes in the level of pseudo-cholinesterase in the plasma of male rats do not entail similar changes in the level of true cholinesterase in the plasma.   Changes in the level of true cholinesterase in the plasma do not necessarily reflect changes in the level of this enzyme in brain and muscle.   Consequently, the activities of the cholinesterases of plasma cannot serve as an index of the level of true cholinesterase in tissues under pathological conditions.

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NUTRITIVE VALUE OF FRUCTOSE FOR RATS AND EFFECTS PRODUCED ON ITS UTILIZATION BY THIAMINE[1]

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IN PREVIOUS experiments the single food choice technique was used to determine the nutritive value of glucose and sucrose and the effects produced on their utilization by thiamine (1, 2). Rats of a standard weight were placed on a diet consisting of only one sugar, either glucose or sucrose. The length of time that the rats survived was taken as a measure of the nutritive value of the sugar. Likewise when the rats had access also to a 0.02 per cent solution of thiamine hydrochloride, the increase in the survival time was taken as a measure of the effects produced by this vitamin on the utilization of the sugar. On either glucose or sucrose the rats survived an average of 37 days. When a thiamine solution was available the rats drank it freely and their average survival time on glucose increased to 74 days and on sucrose to 56 days.

These experiments indicated that thiamine has much less effect on the utilization of sucrose than it does on glucose. It was suggested that the presence of the fructose moiety might explain the reduced effect of thiamine on the utilization of sucrose. The following single food choice experiments on fructose were undertaken to test this suggestion.

METHODS

Female rats, albino or hooded, weighing between 80 and 90 grams were placed in separate activity cages, each equipped with a living compartment and a revolving drum (3). The living compartment contained a non-spillable food-cup and one 100 cc. graduated inverted water bottle. For the next 15 to 20 days, that is until the rats weighed between 120 to 149 grams, they ate a stock diet[2] and drank tap water. Then in one series of experiments the stock diet was replaced with granulated fructose (C.P. Special, Pfanstiehl Chemical Company) and in a second series the stock diet was replaced with a 40 per cent solution of fructose. In the thiamine experiments, the rats had access at the same time to a 0.02 per cent solution of thiamine hydrochloride.

Records were made daily of the food and fluid intake, running activity, as measured by the number of revolutions of the drum, and of vaginal smears. The rats were weighed at weekly intervals. Inspections were made at frequent intervals

for signs of nutritive deficiency; all noticeable changes were recorded and photo-graphed.

<div align="center">RESULTS</div>

Survival Times.   Figure 1 summarizes the results.   It gives the mean survival times (solid lines) and the standard error of the mean (dotted lines) for the rats that received fructose (granulated) without and with access to the thiamine solution. For comparison, it gives the mean survival times for the rats that in previous ex-periments received glucose or sucrose without and with access to thiamine.   The graph shows also the percentage increase in survival times of the rats that had access to thiamine over those that received only the sugars.

Single Foods—Fructose.   On granulated fructose alone the rats lived longer than they did on either glucose or sucrose.   The survival times of the 15 rats on the granu-lated fructose averaged 45.3 days, which is 8 and 9 days longer than the average for
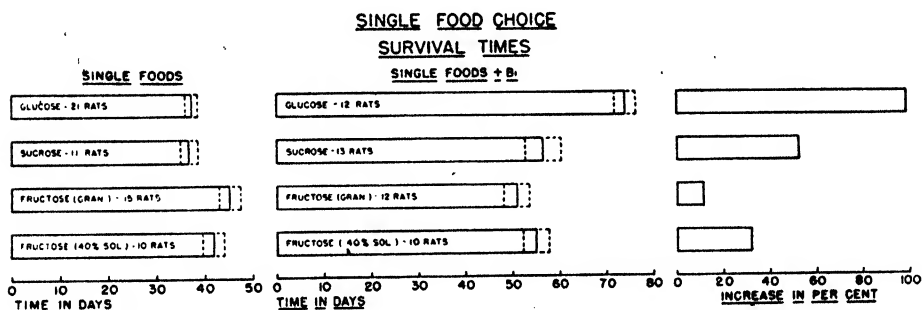
Fig. 1. CHART SHOWING THE AVERAGE SURVIVAL TIMES in days of rats on the single foods without and with access to a 0.02 per cent solution of thiamine hydrochloride; also the increase in per cent produced on the survival times by the thiamine hydrochloride.

glucose (powdered) and sucrose (granulated) respectively.   In contrast to the rats on glucose or sucrose that at no time showed any signs of nutritional deficiency, ex-cept for emaciation, the rats on fructose showed a marked loss of hair, particularly on the head and over the haunches.   The hair, when pulled, came out very easily and in large tufts.   The general effect was not unlike that produced by a dietary de-ficiency of biotin.

It appeared at first that this marked loss of hair resulted from some metabolic effect of the fructose and that, in spite of the longer survival times, as compared to those of the rats on glucose or sucrose, it represented a definite nutritional deficiency. The results of further observations indicated, however, that the loss of hair may de-pend on an external rather than an internal action of the fructose.   It was found that the hair of these animals was sticky to the touch; likewise the wire mesh of the living compartment and revolving drums was sticky.   Apparently in some way during eating, the sugar stuck to the hair around the snout or to the paws and from there was distributed to the hair and to the cage, or indirectly from the wire to the hair. In an attempt to eliminate this widespread distribution of sugar to the hair, fructose

was offered to the rats in a 40 per cent solution rather than in granulated form. This meant that the rats could ingest the sugar without getting it on their paws.

Figure 1 shows that the 10 rats on the fructose solution survived on the average of 41.8 days, approximately as long as did the rats on the granulated fructose; definitely longer than did the rats on glucose or sucrose. In marked contrast to the rats on the granulated fructose they showed either no loss of hair at all, or only very slight loss, and in most instances their hair showed no signs of being sticky. Their cages showed only a very slight stickiness, or none at all.

Granulated Fructose and B₁. Figure 1 shows that the 12 rats on fructose with access to the 0.02 per cent solution of thiamine hydrochloride survived on the average 50.7 days, only 6 days longer than on the granulated fructose alone, representing only a 10.6 per cent increase. Like the rats on granulated fructose alone, these
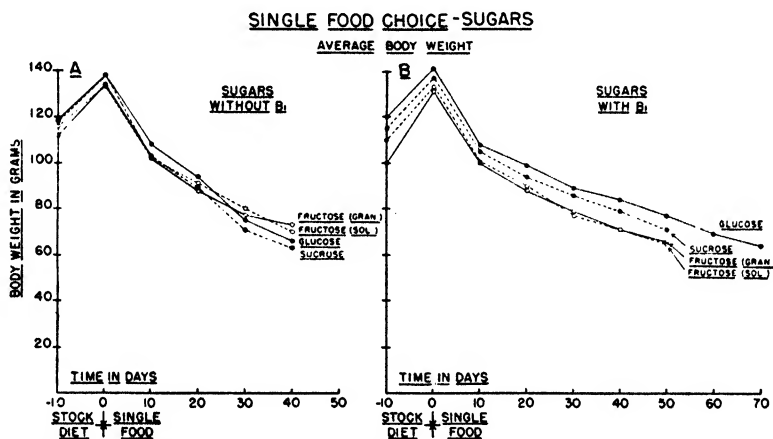
Fig. 2. GRAPHS SHOWING THE AVERAGE BODY WEIGHTS of the rats on the single food choice diets.

rats showed a marked loss of hair over the head and haunches and the presence of a sugary film on the remaining hair.

Fructose Solution and B₁. The 10 rats on the 40 per cent solution of fructose and with access to the B₁ solution survived on the average 55.1 days, not significantly longer than did the rats on the granulated fructose and B₁. The hair of these rats was not sticky; nor was there any hair loss. The cages showed only a slight tendency to stickiness.

A comparison of the percentage increase in survival times of the rats with access to B₁, over those that had the sugars only, shows a marked difference between the three sugars. With access to B₁ the rats fed glucose showed a 98.9 per cent increase in survival time; the rats on sucrose a 52.1 per cent increase; the rats on granulated fructose a 10.6 per cent increase; and the rats on fructose solution a 31.8 per cent increase.

Body Weight—Fructose. Figure 2 summarizes the results. The curves give the average body weights on the 10th day before the start of the single food diets, the day on which the diet started and, also, the successive 10-day periods. On the single foods without B₁ the curves are much the same, but on the 40th day the rats

on the granulated fructose and those on the fructose solution weighed slightly more than did the rats on either glucose or sucrose. They also lived longer.

Fructose and B_1. With access to the thiamine solution the rats lost weight at a slower rate than did the rats without access to this vitamin (fig. 2B). The rats on glucose lost weight at a slower rate than did those on fructose (granulated or in solution). Here again a direct relationship existed between body weight and survival time.

Food Intake—Fructose. Figure 3 gives the average daily food intake in cal/kg. of body weight for the rats on the three sugars without and with access to thiamine. Figure 3A shows that on the stock diet the average daily intake of the four groups of rats ranged from 368 to 419 cal/kg. During the first few days on the single food diets, the rats ate about the same amount of glucose as they had previously eaten
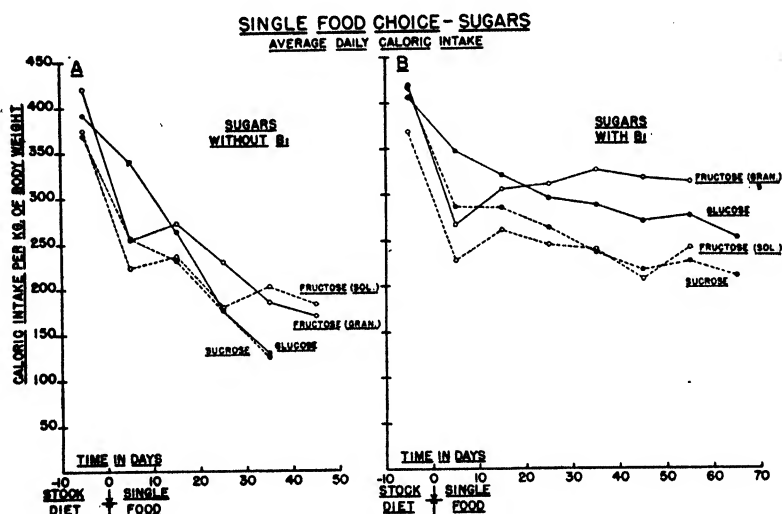
Fig. 3. GRAPHS SHOWING THE AVERAGE DAILY INTAKE in cal/kg. of the rats on the single food choice diet.

of the stock diet; in marked contrast they ate very little or no sucrose or fructose. For the first 10 days the average intake dropped only slightly for the rats on glucose (from 392 to 340), but much more for the rats on sucrose (from 393 to 265) or on fructose (from 419 to 267). Later the rats began to eat more sucrose and fructose and to some extent make up for the initial refusal. The intake of the rats on glucose decreased at a steady rate while that of the rats on fructose showed an increase during the second 10-day period. During the 30- to 40-day period the rats on fructose took much larger amounts than did the rats on either glucose or sucrose. This higher food intake might thus account for the longer survival times and higher average body weights of the rats on fructose as compared to those of the rats on sucrose or glucose.

Fructose and B_1. With the thiamine supplement, just as without, the rats on sucrose and fructose ate very little or no sugar during the first few days after the

change from the stock diet.   Figure 3B shows that this brought the average daily intake of these two sugars for the first 10-day period far below that of the rats on glucose.   During the next 10-day period the rats on sucrose and fructose either increased their intake or maintained it at the same level.   In the subsequent 10-day periods the average of the rats on sucrose, fructose (solution) or glucose decreased at a slow rate, while that of the rats on fructose (granulated) continued to show a very slight increase.   In all instances the daily intake levels were far above those of the rats on the sugars without the thiamine supplement.   The record for the 50- to 60-day period shows that the intake was lowest for the rats on sucrose
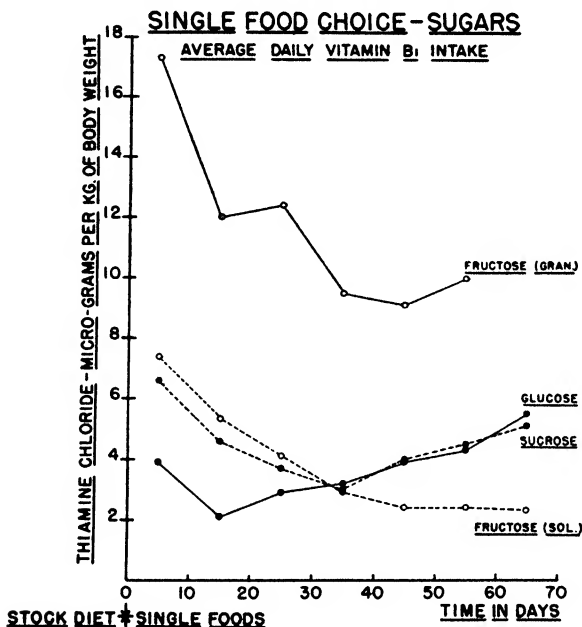
Fig. 4. GRAPH SHOWING THE AVERAGE DAILY INTAKE of thiamine hydrochloride on the regular food choice diet.

and fructose (solution); next came glucose; then fructose (granulated).   The actual consumption of granulated fructose was not, however, so high as this figure would indicate, since a considerable amount was diverted to the rats' hair and skin and to the wires of the cages.   Leaving out the record of the rats on the granulated fructose the results indicate that the survival times varied with the caloric intake.

Thiamine Intake.   Figure 4 summarizes the results.   It gives the average daily intake of thiamine hydrochloride per kilogram body weight for each group of rats. The curve for fructose in solution parallels that for sucrose during the first 30 days, with both decreasing gradually; thereafter the rats on fructose solution continued to take less and less thiamine while those on sucrose gradually increased their intake, now coinciding with the thiamine intake for the rats on glucose.   The glucose rats started out taking much less thiamine than the others and then after an initial drop increased their intakes steadily.

The groups of rats on granulated fructose took very much more thiamine throughout their lives than did any other group. Their curve also shows a gradual though irregular decline. The presence of the large amounts of fructose on the hair and on the cages may in some way account for this higher thiamine intake.

Vaginal Smears. No difference in the estrous cycles as determined by the vaginal smears was found between the three sugars. In all instances only one or two four-day cycles were found after the start of the single food diet, without and with B_1. After that the rats all showed constant diestrous smears.

DISCUSSION

The results showed that when granulated fructose is fed as a single food to rats it sticks to their paws and snouts and from there becomes distributed to the hair and skin on the rest of their bodies, also to the wires of their cages. This same tendency was shown by sucrose, but to a much less marked degree; it was not shown at all by glucose.

This finding indicates that fructose must have some property that is definitely less marked or even lacking in glucose. Fructose is about twice as soluble as sucrose (374.78 gm/100 gm. of water as compared to 203.99) and about four times as soluble as glucose (97.51 gm/100 gm. of water) (4), so this property could be a higher solubility. However it absorbs moisture much more readily than does glucose, so this property more likely is a higher hygroscopic action.
property involves physical characteristics other than these two.

The presence of fructose on the skin and hair introduces several complications in these experiments. In the first place it may enable bacteria or moulds to grow on the hair and in the follicles and so do damage to the hair; in the second place bacteria or moulds thus nourished may serve as a source of nitrogenous substances and vitamins that the rats may obtain as they lick their hair and skin in cleaning themselves; and in the third place the bacteria thus ingested might change the flora of the digestive tract. It would not explain the failure of thiamine to have as much effect on the utilization of fructose as it does on glucose.

The fact that the rat's hair grows in waves that start from the belly and move up the sides in more or less parallel lines to the back and reach last of all the top of the head and the haunches (5) may explain the localization of hair loss in these places in the present experiment. Any hair lost over these areas would be the last to be replaced.

CONCLUSIONS

These single food choice experiments showed that fructose has some property that makes it stick to the paws, snout, hair and skin of rats. The presence of the sugar on the hair in some way promotes the loss of hair especially on the head and haunches. The evidence indicates that the sugar achieves this effect through an external rather than an internal action. Single food choice experiments on fructose are complicated 1) by the fact that not all of the fructose taken from the food receptacles is actually eaten by the rats, some of it being diverted to the skin and hair, and the wires of the cages; and 2) by the possibility that bacteria or moulds may grow

·in the fructose and thus supply the rats with vitamins and proteins. Offering the fructose in solution at least in part obviated these complications. Under the conditions of these single food choice experiments the rats lived longer on fructose (solution) than on either glucose or sucrose (41.8 days as compared to 37.2 days and 36.8 days respectively). Access to a 0.02 per cent solution of thiamine hydrochloride increased the survival times of the rats on fructose to 55.1 days, on sucrose to 56.4 days, on glucose to 74.0 days, or 31.8, 52.1 and 98.9 per cent, respectively.

These results indicate that thiamine has less effect on the utilization of sucrose and fructose than it does on the utilization of glucose.

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EFFECT OF CERTAIN CHOLERETIC AGENTS ON EXCRETION OF PIGMENT AND BROMSULFALEIN IN BILE

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IN A previous communication (1) we reported observations on the rate of biliary excretion of endogenous and exogenous bile-pigment and bromsulfalein in Thomas-type tubulated duodenal-fistula dogs (2–4), in which normal nutrition and liver function can be maintained for many months to several years. These data serve as controls for the present study of the influence of certain choleretic agents upon biliary excretion of the substances mentioned. There is comparatively little precise information on these points in the literature. Practically none of the pertinent reported studies is entirely satisfactory because of defects inherent either in the analytical methods employed or in the conditions of the experiment. The majority were acute experiments conducted under anesthesia, which affects the flow of bile and, conceivably, the response to a choleretic agent. Others were performed in dogs with the Rous-McMaster type of permanent external bile fistula, which have been found to have, almost invariably, some degree of impairment of liver function, even though apparently healthy (4, 5).

It is felt that the data presented here, to which these objections cannot be raised, represent the response to administration of the choleretic agents employed under as nearly normal conditions as can be attained at the present time.

Materials and Methods

Five trained, cholecystectomized dogs were used, provided with gastric and duodenal fistulae fitted with large cannulae, as described by Thomas (2). The duodenal fistula was placed opposite the ampulla of Vater and bile was collected by inserting a temporary glass cannula (3, 4) into the common duct. The bile was allowed to drain into graduated tubes until the flow became constant. It was then collected in 15-minute samples.

After a one-hour control bile collection period, the choleretic agents employed were injected intravenously as follows: a) sodium dehydrocholate, 10 cc. of a 20 per cent solution; b) sodium salt of 2-phenylquinoline-4 carboxylic acid (sodium cinchophen), 10 per cent solution, 40 or 50 mg/kg. body weight; c) sodium cholate, 10 per cent solution, 50 mg/kg. body weight.

Bilirubin (Eastman Kodak Co.) was injected intravenously in one per cent Na_2CO_3 solution (one mg. bilirubin/cc.), in a dosage of one mg/kg. body weight. Bromsulfalein (BSP) was injected in a dosage of 5 mg/kg. body weight. These were injected simultaneously with the choleretic agents.

Bile was collected subsequently in 15-minute samples. Each sample was made up to 5 or 10 cc. with distilled water. Determinations of total pigment in bile were made by the method of Malloy (6) and of BSP by a method described by Cantarow and Wirts (7).

RESULTS

Endogenous Pigment Excretion (tables 1 and 2). The bile volume increased promptly in 2 dogs after intravenous injection of 2 gm. of sodium dehydrocholate,

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reaching a maximum in 30 to 60 minutes and returning to the pre-injection level in 1½ to 2½ hours. The hourly volume increased 271 and 854 per cent in the first hour, and 0 and 297 per cent in the second hour. There was a decrease of 78 and 29 per cent during the third hour. The net three-hour increase was 193 and 1122 per cent, respectively. The bile pigment excretion increased promptly, reaching a maximum in the first 15 minutes, the concentration of pigment falling to a minimum value at 30 minutes. The changes in pigment excretion were as follows: first hour, +90 and

TABLE 1. EFFECT OF CHOLERETIC AGENTS ON BILE VOLUME AND BILE PIGMENT OUTPUT

DOG	CHOLERETIC AGENT	PRE-INJECTION BILE 1 HOUR		POST-INJECTION BILE					
				1st Hour		2nd Hour		3rd Hour	
		Vol.	Pigment	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment
		mg./ kg.	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*
3	Sodium dehydrocholate	100	3.5	2.72	33.4	4.15	13.9	2.94	2.5	2.10
5	Sodium dehydrocholate	144	14.0	2.15	52.0	4.10	13.8	2.43	3.1	2.10
1	Sodium cinchophen	50	1.4	2.80	9.4	7.31	6.2	4.83	5.2	7.27
3	Sodium cinchophen	50	2.1	1.87	24.8	8.06	21.6	6.20	20.4	5.35
4	Sodium cinchophen	50	8.2	2.06	21.2	3.78	15.0	4.68	0.8	0.46
5	Sodium cinchophen	40	9.0	2.74	36.0	4.55	18.2	2.61	8.1	1.79
5	Sodium cinchophen	40	3.3	1.00	28.3	8.42	12.7	3.13	5.4	3.02
2	Sodium cholate	50	3.3	2.59	8.5	4.20	0.9	0.67	1.1	4.13
2	Sodium cholate	50	2.8	2.93	13.7	5.46	7.2	6.88	3.4	4.06
5	Sodium cholate	50	7.4	2.17	14.0	3.9ⁿ	6.2	4.25	6.4	5.92

TABLE 2. EFFECT OF DOUBLE INJECTION OF SODIUM CINCHOPHEN (EACH 50 MG/KG. BODY WEIGHT) ON BILE VOLUME AND BILE PIGMENT OUTPUT

DOG	PRE-INJECTION 1 HOUR		AFTER FIRST INJECTION 1 HOUR		AFTER SECOND INJECTION			
					1st Hour		2nd Hour	
	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment
	cc.	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*
3	5.5	3.10	25.6	5.92	24.4	3.94	21.3	3.62
5	5.5	2.28	27.2	5.05	18.1	1.92	17.2	2.31
5	10.5	3.18	43.0	4.70	38.6	3.98	32.8	4.43

+53 per cent; second hour, 0 and +13 per cent; third hour, −23 and 0 per cent; the net three-hour increase was 67 and 66 per cent respectively.

In five instances after injection of sodium cinchophen the bile volume increased to a maximum in 15 to 30 minutes, the choleresis persisting for 1¼–3+ hours. The hourly changes were as follows: first hour, +158 to +1081 per cent; second hour, +83 to +929 per cent; third hour, −90 to +871 per cent. The net three-hour volume increase was 151–2881 per cent. The bile pigment excretion rose promptly to a maximum within 15 minutes in 4 instances and at 90 minutes in 1 instance. The concentration of pigment fell simultaneously to a minimum level in 30 to 60 minutes. The hourly changes in pigment excretion were as follows: first hour, +66

to +742 per cent; second hour, o to +231 per cent, third hour, −78 to +202 per cent. The net three-hour increase was 31–1157 per cent.

In three instances after injection of sodium cholate, the bile volume rose to a maximum in 15 to 45 minutes, returning to the control level in 30 to 90 minutes. The hourly volume changes were as follows: first hour, +87 to +389 per cent; second hour, −73 to +157 per cent; third hour −67 to +21 per cent. The net three-hour volume increase was 17 to 567 per cent. The bile pigment excretion rose to a maximum within 15 minutes, the pigment concentration falling to a minimum level in 30 to 45 minutes. The hourly pigment changes were as follows: first hour, +62 to +86 per cent; second hour, −74 to +135 per cent; third hour, +38 to +173

TABLE 3. EFFECT OF CHOLERETIC AGENTS ON BILIARY EXCRETION OF EXOGENOUS BILIRUBIN (1 MG/KG. BODY WEIGHT)

DOG	CHOLERETIC AGENT	PRE-INJECTION 1 HOUR			POST-INJECTION									Total recovery
					1st Hour				2nd Hour				
			Pigment			Pigment				Pigment			
		Vol.	Max. conc.	Total	Vol.	Max. conc.	Total	Recovery	Vol.	Max. conc.	Total	Recovery	
		mg./kg.	cc.	mg.%	mg.	cc.	mg.%	mg.	%	cc.	mg.%	mg.	%	%
3	Sodium dehydrocholate	100	6.6	68.3	2.69	39.1	61.4	14.5	59.3	16.6	47.9	6.6	19.4	78.7
5	Sodium dehydrocholate	144	8.5	60.0	2.89	55.1	36.4	13.8	77.8	16.4	52.9	4.7	13.1	90.9
3	Sodium cinchophen	50	15.2	35.3	3.45	22.9	111.4	16.1	63.4	21.6	27.8	5.5	10.2	73.6
3	Sodium cinchophen	50	2.7	161.6	4.28	22.3	142.5	20.4	80.7	20.1	29.8	5.4	5.8	86.5
4	Sodium cinchophen	50	1.6	92.5	1.52	10.3	107.2	9.4	79.0					79.0
2	Sodium cholate	50	8.1	43.9	2.61	16.2	94.4	10.6		1.5	1075.0	4.7		
3	Sodium cholate	50	4.1	108.3	4.18	16.5	93.6	10.6		4.9	293.4	7.7		

per cent. The net three-hour increase was 47 to 350 per cent. Hemoglobinemia and hemoglobinuria were present in every case.

In three experiments in which a second dose of sodium cinchophen was injected at the height of the choleretic response to the first injection, the volume changes were as follows (table 2): first hour after first injection, +309 to +395 per cent; first hour after second injection, +230 to +344 per cent; second hour after second injection, +212 to +287 per cent. The net three-hour volume increase was 789 to 996 per cent. The pigment changes were as follows: first hour after first injection, +48 to +121 per cent; first hour after second injection, −16 to +27 per cent; second hour after second injection, o to +39 per cent. The net three-hour pigment increase was +105 to +135 per cent.

Exogenous Pigment Excretion (table 3). The percentage recovery of injected bilirubin was calculated on the basis of the amount of pigment excreted after injection

in excess of that excreted during the pre-injection period. In view of the data presented in table 1, indicating an increase in biliary excretion of endogenous pigment following administration of these choleretic agents, the validity of this method of calculation is questionable. Because of the observed variability of this effect of these agents in the same animal on different occasions, it is impossible to apply a correction factor that is entirely satisfactory. However, if one employs a factor representing the average increment in biliary pigment during the first and second hours following injection of each choleretic agent alone (table 1), the corrected recovery values are as follows: after sodium dehydrocholate, first hour, 50.6 and 65.9 per cent; second hour, 18.3 and 11.1 per cent; total, 68.9 and 77.0 per cent. After sodium cinchophen: first hour, 35.2 to 58.9 per cent; second hour, 0 to 5.4 per cent; total, 35.2 to 53.5 per cent. Recovery of exogenous bilirubin was not calculated in animals receiving sodium cholate because of the occurrence of hemolysis and the attendant increased production of bilirubin.

After sodium dehydrocholate, maximum pigment excretion was attained at 15 to 30 minutes and maximum choleresis at 30 to 60 minutes. After sodium cinchophen, maximum pigment excretion occurred within 15 to 45 minutes and maximum choleresis at 30 to 75 minutes.

Bromsulfalein Excretion. The pertinent data are presented in table 4. The effect of the choleric agents in increasing bile pigment excretion was not affected significantly by simultaneous administration of bromsulfalein. Dye appeared in the bile within the first 15 minutes in every instance. After sodium dehydrocholate, maximum BSP excretion was attained at 75 minutes, maximum concentration of BSP in the bile at 90 minutes and maximum choleresis at 30 minutes. After sodium cinchophen, maximum BSP excretion and concentration were attained at 30 to 45 minutes and maximum choleresis at 30 to 75 minutes. After sodium cholate, maximum BSP excretion and concentration were attained at 30 to 75 minutes and maximum choleresis at 15 to 30 minutes.

<div align="center">COMMENT</div>

The data presented here are not sufficient to justify any definite conclusions regarding the choleretic effect of the agents employed. However, certain observations seem worthy of mention, particularly in view of the fact that practically all previously reported data bearing on this matter were obtained under conditions in which hepatic function cannot be accepted as normal, i.e. in acute experiments in anesthetized animals or in dogs with permanent external biliary fistulae (4, 5). In general, the increase in bile volume was greater and more prolonged after injection of sodium cinchophen than after much larger doses of sodium dehydrocholate and the latter was more effective than sodium cholate. In the case of each agent, the magnitude of the choleretic response was roughly in inverse proportion to the basal bile volume, i.e. the lower the control rate of flow, the greater the choleretic response.

The striking increase in bile flow during the first hour after injection of sodium dehydrocholate (tables 1 and 4) was accompanied by a moderate increase in pigment excretion, which fell during the second hour to approximately the control level despite persistence of some degree of choleresis in 2 of 4 animals. In each instance

the augmented pigment excretion could be accounted for on the basis of flushing of the 'dead space' of the bile duct system, estimated to be about 5 cc. (1), by the accelerated flow of bile. The findings were somewhat different after a single injection of sodium cinchophen (tables 1 and 4). The increase in pigment output during the first hour generally exceeded that produced by dehydrocholate, despite the usually less marked increase in bile volume. Moreover, except in one instance (*dog 5*, table 1), the increase in pigment persisted during the prolonged period of choleresis and did not appear to be quantitatively explicable on the basis of flushing of the bile duct system of its pre-injection pigment content.

The effect of various choleretic agents on the output of pigment in the bile is disputed in the literature (8–12). The data reported here are in accord with the view

TABLE 4. EFFECT OF CHOLERETIC AGENTS ON BILIARY EXCRETION OF BROMSULFALEIN (5 MG/KG. BODY WEIGHT)

DOG	CHOLERETIC AGENT	PRE-INJECTION 1 HOUR		POST-INJECTION									
				1st Hour		2nd Hour		3rd Hour		% B S P Recovery			
		Vol.	Pig-ment	Vol.	Pig-ment	Vol.	Pig-ment	Vol.	Pig-ment	1st hr.	2nd hr.	3rd hr.	Total
		mg./kg.	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	%	%	%	%
3	Sodium dehydro-cholate	100	5.9	3.43	38.3	5.16	18.5	4.57	6.5	3.25	26.3	36.7	7.0	70.0
5	Sodium dehydro-cholate	144	16.0	1.36	47.3	3.97	16.0	1.84	4.4	0.39	20.3	25.2	6.2	51.7
3	Sodium cincho-phen	40	8.4	3.32	32.9	4.22	26.1	4.41	26.7	4.84	67.7	7.6	2.6	77.9
5	Sodium cincho-phen	40	7.9	2.69	31.2	4.54	20.2	4.66	10.8	3.19	49.9	10.3	1.6	61.8
5	Sodium cincho-phen	40	2.7	2.04	11.5	4.60	22.8	6.19	11.1	4.02	40.3	25.2	5.9	71.4
2	Sodium cholate	50	7.0	4.81	10.8	16.22	5.7	6.24	2.8	0.52	21.6	8.3	0.7	30.6
3	Sodium cholate	50	2.8	2.68	13.3	7.27	9.1	14.79	4.6	2.80	7.3	14.8	2.8	24.9
5	Sodium cholate	50	6.8	1.42	16.0	7.92	8.6	2.51	7.9	0.83	11.3	3.6	1.2	16.1

that sodium dehydrocholate does not cause increased excretion of endogenous pigment by the hepatic cells. The observations referred to above suggest that sodium cinchophen, on the other hand, may actually increase hepatic excretion of endogenous pigment as a part of its choleretic effect. However, this view is not supported by the data obtained with a double injection of sodium cinchophen (table 2). Under these circumstances, in only one of three instances was the well-sustained increase in bile flow after the second injection accompanied by an increase in pigment excretion that could not be attributable to flushing of the bile ducts. No satisfactory explanation is afforded for this apparent discrepancy.

The findings after injection of sodium cholate cannot be interpreted in terms of the effect of this agent on biliary excretion of endogenous pigment because of the consistent occurrence of hemoglobinemia in these animals. The observed increase in pigment excretion under these circumstances is in accord with previous reports (1, 13).

In a previous study (1) it was found that the maximum output of pigment in the bile occurred 30 to 60 minutes after intravenous injection of one mg. bilirubin /kg. of body weight; 27.6 to 65 per cent of the injected bilirubin was recovered as 'extra' bile pigment during the first hour and 6.6 to 37.9 per cent during the second hour (total 2-hr. recovery, 42.3–75.6%). Even though the exogenous pigment recovery values calculated in the present report must be regarded as only approximate, they are in substantial agreement with these control figures. It would appear that sodium dehydrocholate and sodium cinchophen neither accelerate nor retard the biliary excretion of exogenous bilirubin under the existing experimental conditions. A similar conclusion was reached by Berman *et al.* (14) regarding the effect of sodium dehydrocholate on the excretion of larger quantities of intravenously injected bilirubin (5 and 12 mg/kg.) in anesthetized dogs. However, their recovery values were considerably lower than those reported here.

In control studies (1), 41.7 to 69.8 per cent of intravenously injected BSP was excreted in the bile during the first hour, 7.4 to 19.8 per cent during the second hour and 1.6 to 9.8 per cent during the third hour (3-hr. total, 55.6–96.9%). Maximum dye excretion occurred at 30 to 45 minutes. The data obtained following administration of sodium cinchophen coincide well with these figures. In the case of sodium dehydrocholate, however, although the total three-hour BSP excretion was of the same order of magnitude as in the control group, the time of maximum excretion was delayed (75 min.) and less was excreted during the first than during the second hour. This suppression of BSP excretion during the period of maximum choleresis induced by dehydrocholate is in accord with previously reported observations in dogs with permanent external bile fistulae (15).

Administration of sodium cholate, in sharp contrast to the other two agents employed, was accompanied by considerable reduction in the three-hour excretion of BSP. Whereas in the case of sodium dehydrocholate biliary excretion of the dye was delayed only temporarily, increasing as choleresis subsided, this was not true in animals receiving sodium cholate. It is difficult to explain this phenomenon on any basis other than impairment of the BSP-excreting function of the liver, either by the sodium cholate or by the hemoglobinemia that attended its administration. It is interesting in this connection that Grodins *et al.* (16) found that intravenous injection of sodium dehydrocholate and of sodium cinchophen was followed by an increased hepatic arterial blood flow whereas sodium cholate produced no such effect.

SUMMARY

The effect of sodium dehydrocholate, sodium cinchophen and sodium cholate upon the rate of bile flow and biliary excretion of endogenous and exogenous bilirubin and of bromsulfalein was studied in cholecystectomized dogs provided with gastric and duodenal fistulae fitted with large cannulae.

The increased bile flow induced by these agents was accompanied by an increase in bile pigment excretion. In the case of sodium cholate, this was attributed to the attendant intravascular hemolysis and consequent hemoglobinemia. In the case of sodium dehydrocholate, the increased pigment output could be attributed to flushing of the 'dead space' of the bile duct system of its pre-injection pigment content. This phenomenon did not appear to afford an adequate explanation for

the augmented pigment output following administration of sodium cinchophen. Although the evidence is inconclusive on this point, it suggests that this agent may actually increase hepatic excretion of endogenous pigment as a part of its choleretic effect.

Biliary excretion of exogenous bilirubin was neither accelerated nor retarded by simultaneous intravenous injection of either sodium dehydrocholate or sodium cinchophen. Biliary excretion of BSP was not affected by sodium cinchophen but was delayed somewhat by sodium dehydrocholate, being temporarily suppressed during the period of maximum choleresis and increasing subsequently. The total three-hour excretion was within control limits. After administration of sodium cholate the three-hour excretion of BSP was reduced to about 50 per cent or less of the control values. This apparent impairment of hepatic function may be an effect of the cholate itself or of the hemoglobinemia that followed its administration.

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CHOLINE AND THE PRODUCTION OF POLYCYTHEMIA BY COBALT IN THE RAT [1]

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THE production of polycythemia by the oral administration of a small amount of cobalt was first demonstrated in an experimental animal, the rat, in 1929. Subsequently, this observation has been corroborated by other workers both in the rat and in several other species of animals.

A number of substances have been administered with cobalt in order to determine their effects on the production of polycythemia. Marshall (1) claimed that the injection of certain liver extracts would cause a temporary fall in the red blood cell count of rats rendered polycythemic by cobalt. Davis (2) reported that the feeding of 75 gm. per day of raw beef or hog liver to dogs with cobalt polycythemia caused a reduction in the red blood cell count. The depression was maintained for four weeks despite the continued daily administration of cobalt. The erythrocyte counts returned to polycythemic levels within four days after the liver feeding was discontinued.

In an attempt to determine the substance in liver responsible for the inhibition of the hemopoietic effect of cobalt, choline was investigated. Davis (3) reported that the oral administration of 8 mg. of choline chloride per kilo body weight to dogs receiving cobalt resulted in a prompt reduction of the red blood cells to the extent of 15 to 20 per cent and, within three days, a return to normal levels. Upon cessation of choline administration, the hemoglobin values returned to polycythemic levels. Leukocyte counts did not change throughout the experiment. The same dose of choline chloride given to normal dogs did not change the red blood counts.

In view of the foregoing observations, the present study was undertaken to determine the effects of the oral administration of choline on the development of polycythemia in the rat receiving cobalt.

EXPERIMENTAL

Male, weanling albino rats, weighing 42 to 50 gm., of the Connecticut Agricultural Experimental Station were used. The rats were given a synthetic basal diet having the following percentage composition: casein, 20.0; sucrose, 10.0; white corn dextrin, 40.0; Crisco, 25.6; and Wesson's salt mixture, 4.0. The synthetic vitamin supplements were incorporated in the foregoing basal diet in the following amounts

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(in mg/100 gm. of diet): thiamin, 1; riboflavin, 2; pyridoxine, 1; niacinamide, 2; calcium pantothenate, 4; inositol, 200; p-amino benzoic acid, 60; folic acid, 2; biotin, 0.001; and 2 methyl 1–4 naphthoquinone, 0.4. In addition, vitamins A, D, and E were supplied as haliver oil with viosterol fortified with alpha-tocopherol (100 mg/ 50 cc). Three drops were administered to each rat twice weekly. To this synthetic basal diet various supplements for the different experimental groups, as described below, were added.

TABLE 1. AVERAGE BODY WEIGHTS[1] (IN GM.) OF CONTROL RATS AND OF RATS GIVEN COBALT WITHOUT AND WITH CHOLINE

WKS. ON EXPER.	GROUP			
	Control	Cobalt Alone	Cobalt + 'Low' Choline[2]	Cobalt + 'High' Choline[2]
0	44 (38– 50)	45 (37– 52)	46 (38– 58)	52 (45– 62)
1	110 (84–143)	77 (60– 95)	71 (65– 78)	83 (71– 96)
2	162 (145–203)	98 (80–115)	102 (92–118)	86 (72– 98)
3	209 (158–253)	113 (93–130)	112 (94–134)	97 (81–113)
4	258 (195–310)	135 (112–165)	132 (113–168)	117 (94–138)
5	298 (240–367)	157 (128–203)	155 (131–208)	136 (107–162)
6	325 (275–405)	176 (145–230)	173 (148–238)	166 (134–197)
7	352 (285–453)	195 (160–255)	190 (165–253)	188 (149–218)
8	376 (298–495)	205 (181–258)	201 (178–260)	205 (163–237)
9	398 (318–525)	222 (190–280)	218 (187–283)	222 (174–253)
10	420 (330–553)	237 (198–303)	233 (198–292)	241 (189–279)
11	442 (343–586)	253 (204–325)	249 (220–310)	252 (203–295)
12	463 (353–626)	269 (210–335)	260 (213–343)	266 (212–313)
13	485 (363–660)	285 (214–360)	272 (233–372)	[3]
14	493 (361–675)	289 (208–360)	277 (244–370)	
15	503 (370–685)	301 (225–375)	281 (248–372)	
16	517 (380–698)	309 (228–390)	288 (250–382)	
17	520 (375–704)	314 (225–400)	293 (260–383)	
18	526 (390–698)	326 (240–416)	305 (265–385)	
19	535 (398–702)	333 (240–419)	318 (258–408)	
20	542 (410–706)	347 (260–433)	336 (265–404)	

[1] Minimum and maximum values for individual animals are given in parentheses.
[2] 'Low' level of choline = 2.0 gm kilo diet; 'High' level = 6.0 gm kilo diet.
[3] Group discontinued.

The animals were divided into four groups, the control group, the cobalt group, and two groups receiving cobalt plus choline. Choline was given at two different levels: 2.0 gm. (Davis used 1.91 gm. per kilo diet), and 6.0 gm/kilo diet, respectively. The higher level was used to determine what effect would be obtained with a higher dosage. The control animals received 2 gm. of choline per kilo diet. The other three groups were given 477 mg. of recrystallized CoSO$_4$·7H$_2$O per kilo diet. This amount supplies approximately 1.0 mg. cobalt per day. Each group consisted of 12 animals and the experiment was continued for a period of 20 weeks. The body weights of the animals were followed weekly. The food intake was recorded daily and the hemoglobin levels were measured bi-weekly. The hemoglobin values were determined by an acid hematin method employing a Coleman spectrophotometer

calibrated by the O_2 capacity method.   Samples of blood were obtained by piercing the dorsal tail vein of the animal.

RESULTS AND DISCUSSION

From the data in table 1, showing the average body weights for the various groups, it is evident that the control animals grew at a rapid rate reaching a value

TABLE 2.   AVERAGE HEMOGLOBIN VALUES[1] (GM. PER CENT) OF CONTROL RATS AND OF RATS GIVEN COBALT WITHOUT AND WITH CHOLINE

WKS. ON EXPER.	GROUP			
	Control	Cobalt Alone	Cobalt + 'Low' Choline[2]	Cobalt + 'High' Choline[2]
Initial	10.5 (8.5–12.7)	11.5 (8.9–13.7)	11.5 (7.0–13.7)	11.3 (10.0–13.3)
2	12.8 (11.3–15.0)	12.5 (12.6–16.4)	14.8 (12.8–17.4)	15.2 (14.0–16.2)
4	13.3 (11.6–14.4)	15.5 (13.1–19.3)	16.6 (13.7–21.2)	16.1 (13.9–17.7)
6	14.7 (13.3–15.9)	16.0 (14.0–18.5)	17.6 (15.1–19.8)	16.8 (13.5–19.1)
8	14.8 (12.8–16.1)	17.3 (15.5–20.2)	17.6 (15.9–19.9)	18.5 (17.9–19.4)
10	14.8 (13.9–15.8)	18.6 (16.1–20.4)	18.9 (16.6–20.4)	18.9 (17.0–19.6)
12	15.2 (13.4–16.3)	19.0 (16.6–20.8)	19.2 (17.2–20.4)	19.5 (17.6–20.4)
14	15.7 (15.2–16.4)	19.6 (17.4–23.1)	19.6 (16.1–20.4)	3
16	15.6 (15.2–16.2)	19.6 (17.6–22.7)	19.4 (16.8–22.1)	
18	15.6 (14.8–16.1)	19.7 (17.7–22.7)	19.5 (17.3–20.9)	
20	15.5 (14.8–16.3)	20.4 (19.0–22.4)	20.1 (17.4–21.8)	

[1] Minimum and maximum values for individual animals are given in parentheses.

[2] 'Low' level of choline = 2.0 gm kilo diet; 'High' level = 6.0 gm kilo diet.

[3] Group discontinued.

TABLE 3.   STATISTICAL ANALYSIS OF HEMOGLOBIN DATA

GROUP	AV. HEMOGLOBIN	STANDARD DEVIATION	PROBABLE ERROR OF THE MEAN	PROBABLE ERROR OF DIFFERENCE BETWEEN MEANS[1]
Control	15.5	±0.17	±0.08	
Cobalt—12 wks.	19.0	±1.24	±0.24	
Cobalt—20 wks.	20.4	±0.95	±0.23	
Cobalt + 'Low' Choline—20 wks.	20.1	±1.33	±0.37	±0.43
Cobalt + 'High' Choline—12 wks.	19.2	±1.11	±0.25	±0.35

[1] Comparison made with group given cobalt alone for corresponding period of time.

of 542 gm. in 20 weeks.   The animals which received cobalt showed obvious indications of interference with body growth.   Their weight increased steadily but at a far less rapid rate, attaining an average value of only 347 grams in the same experimental period.   The animals of the two groups which received choline with cobalt showed no better growth than those which had received cobalt alone.   Their weights followed a course almost directly parallel with those of the cobalt group, reaching weights of 336 for the low-choline group after 20 weeks and 266 grams at the end of 12 weeks for the high-choline group.   The latter group was discontinued at the end of 12 weeks since their growth and hematological responses had been the same as those of the group given the lower level of choline.   The average daily food intake for the animals

of the various cobalt-fed groups was uniformly between 12 to 14 grams per day; the controls consumed a slightly higher level, 15 to 16 grams.

The average hemoglobin data given in table 2 show the expected steady increase with age in all of the groups. From an average range of initial values of 10.5 to 11.5 grams per cent, the levels of each group increased until approximately the 12th to 14th week of the experimental period, then stabilized. Thus the control rats attained a constant average value of about 15.5 grams per cent, while the cobalt-fed group reached a level of approximately 20 grams per cent. Both groups receiving choline with cobalt also showed an average hemoglobin value of approximately 20 grams per cent. In table 3 are given the results of a statistical analysis of the hemoglobin data. It is evident that there is no significant difference between the values for the animals given cobalt alone and those supplemented with choline at either level.

The present data thus show no demonstrable effect on the polycythemia produced by cobalt from supplements of choline given at either of the dosage levels employed. These data are therefore not in agreement with those obtained by Davis in the dog. It is rather difficult to explain the apparent discrepancy between the results unless it be one of a species difference in response to choline administration. However, Best's report (4) that choline administered orally to the dog does not produce an anemia as has been claimed by other investigators (5, 6) likewise indicates the absence of a demonstrable effect of choline on hemopoiesis in this species at the dosage levels employed. The recent observations of Kunkel and co-workers (7), that the parenteral administration of acetylcholine to dogs does not produce an anemia as has been claimed by others (6), is also interesting in this connection.

CONCLUSIONS

Polycythemia, as evidenced by a marked increase in the hemoglobin level, was produced in rats by the continued oral administration of a small amount of cobalt as a supplement to an adequate, synthetic diet. The cobalt-fed animals grew at a decreased rate and the average hemoglobin level reached approximately 20 gm/100 ml. of blood.

The administration of choline, at a level of either 2.0 or 6.0 gm/kilo diet, with cobalt resulted in the same retardation of growth and the development of a polycythemia to the same extent as found in rats given cobalt alone.

These results are therefore not in accord with the claim that, in the dog, choline prevents the production of polycythemia by cobalt.

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SOME PHYSIOLOGICAL EFFECTS ASSOCIATED WITH CHRONIC CALORIC RESTRICTION[1]

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THE inhibiting effect of caloric restriction on the formation of experimental tumors is well established (1, 2), but little is known concerning the mechanism by which this occurs. As early as 1914 Rous (3) observed that the development of mammary tumor transplants and metastases in the mouse was delayed by food restriction and he suggested that this effect was due to a delay in the vascularization and in the development of a supporting stroma upon which the tumor is dependent. The inhibition of the formation of spontaneous mammary cancer in mice on a reduced food intake has been explained on the basis of a pituitary insufficiency producing a decreased ovarian secretion (4). Thus, one of the factors essential for the occurrence of this type of tumor is deficient when caloric intake is restricted. However, the mechanism of the inhibiting effect of simple caloric restriction on the development of other types of neoplasms remains obscure.

In order to obtain further information on this problem, a preliminary survey of certain organ weights (pituitary, thyroid, adrenal, thymus, ovary, uterus, heart, liver and kidney) was made on groups of 12 to 16 mice kept on *ad libitum* or restricted diets for one week, one month and seven months. The results confirmed the conclusion that a reduction in caloric intake decreased ovarian function and in addition suggested that it increased adrenal cortical activity. In order to obtain further information concerning a possible adrenal stimulation, several criteria that indicate the activity of this organ were investigated. These included data on the weight and ascorbic acid content of the adrenals, the content of glycogen in the liver under different conditions and the activity of the lymphatic system as measured by the weight of the thymus and by lymphocyte counts.

METHODS

Male and female mice of the ABC and Rockland strains, 2 to 3 months of age, were used. Mice of only one strain and one sex were used in any particular experiment. Groups of 6 to 18 mice were kept in screen bottom cages and fed from a special type of feeder (5) either at *ad libitum* (generally 10 to 12 cal/mouse/day) or at restricted levels (6 to 7.2 cal/mouse/day). The diet was weighed into the feeders daily and the mice were fed at about 9:00 A.M. The composition of the artificial type diets is shown in table 1. Water was available to both groups at all times.

Samples for organ weight, liver glycogen, blood sugar and adrenal ascorbic acid determinations were taken from mice as soon as the corneal reflex disappeared following the injection of sodium pentobarbital. Blood was withdrawn from the right ventricle of the exposed heart with a syringe moistened with a solution of the sodium salt of heparin (10 mg/cc). The weights of organs under 50 mg. were quickly determined to 0.02 mg. on a torsion balance. Liver samples were dropped without delay into a tared tube containing 2.0 cc. of 30 per cent alkali and glycogen digests were made according to the method of Good, Kramer and Somogyi (6). Glucose determinations on the digests and on deproteinized blood filtrates were made by the iodometric copper method of Somogyi (7). The liver glycogen values were expressed in terms of the glucose equivalent. The adrenal ascorbic acid determinations were made by the method of Roe and Kuether (8). Tail blood was used for the total white cell counts. The estrus cycle was followed by the usual vaginal smear technique.

TABLE 1. COMPOSITION OF DIETS

	AD LIBITUM	RESTRICTED
Cerelose	78	36.6
Casein	15	15
Corn oil	2	2
Liver conc.	1	1
Salt mix[1]	4	4
	100	58.6

Vitamins added: mg/15 gm. of casein; thiamine hydrochloride, 0.3; riboflavin, 0.3; pyridoxine hydrochloride, 0.3; niacinamide, 0.5; calcium pantothenate, 2.0; inositol, 25; p-aminobenzoic acid, 25; choline chloride, 50; pteroylglutamic acid, 0.1; and biotin, 0.01. Halibut liver oil was added to the corn oil at the level of 7500 vitamin A units and 108 vitamin D units per kg. (6 drops 1 kg. of diet).

[1] PHILLIPS, P. H. AND E. B. HART. *J. Biol. Chem.* 109: 657, 1935.

EXPERIMENTAL AND RESULTS

Liver Glycogen and Blood Sugar. The diurnal fluctuation of liver glycogen and also blood sugar was followed in mice adapted to the restricted and *ad libitum* diets. Since the feeding habits of these mice differed, this variation must be considered in relation to the results. Mice, restricted in food, consumed their daily allotment in less than one hour after the 9:00 A.M. feeding time. However, six hours later their stomachs were still distended with ration, which was mostly in an undigested, semi-dry state. Even 12 hours after feeding time considerable amounts of partially digested food were still present, but by 24 hours the stomach was empty. The mice that were allowed the full ration were also fed at 9:00 A.M., but the major portion of their food was eaten between 6:00 P.M. and midnight. At no time was the stomach distended nor was a dry mass of diet found therein.

After an equilibration period of four weeks or longer, the content of glycogen in the liver was determined in mice taken from the two groups at 3:00 P.M., 9:00 P.M. and 9:00 A.M. the following morning (6, 12 and 24 hours after feeding time, respectively). The results are plotted in figure 1. Each point is an average of 19 or 20 mice in the curve representing the restricted group and of 6 mice to a point in the

curve depicting the mice on the *ad libitum* diet. The same data were replotted for the second 24-hour period to emphasize the cyclic nature of the changes. This diurnal variation in liver glycogen of restricted animals was apparent by the fourth day after caloric restriction was begun and reached the extremes similar to those shown in figure 1 by the tenth day, after which there was no change for as long as 193 days. The unusually high level of liver glycogen in the restricted mice 6 hours after feeding
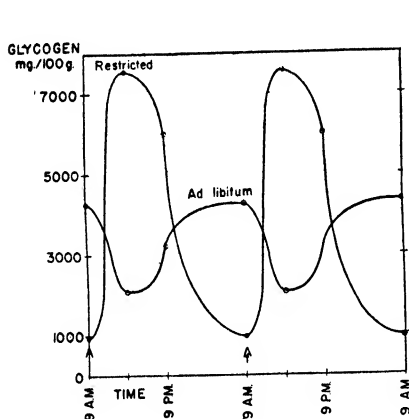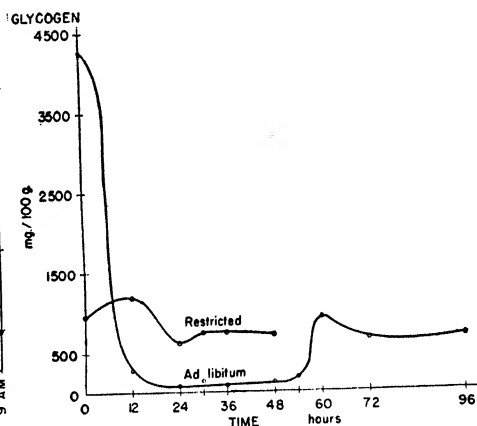
Fig. 1   Fig. 2

Fig. 1 (*left*). DAILY CYCLICAL VARIATION in the amount of glycogen in the livers of mice maintained on *ad libitum* and restricted diets.

Fig. 2 (*right*). EFFECT OF A PROLONGED FAST on the level of glycogen in the livers of mice maintained on *ad libitum* and restricted diets. The last food was given 24 hours prior to the zero time of these curves, and the zero time of this figure corresponds to the points at 9 A.M. on the curves in figure 1. The fast was continued until the death of the animals.

TABLE 2. AVERAGE LEVEL OF BLOOD SUGAR OF MICE ADAPTED TO RESTRICTED AND AD LIBITUM FEEDING HABITS AT 6, 12 AND 24 HOURS AFTER FEEDING TIME. THE NUMBER OF ANIMALS PER GROUP IS SHOWN IN PARENTHESES

TIME	RESTRICTED mg. %	AD LIBITUM mg. %
6	101.4 (7)	114.5 (8)
12	65.1 (6)	91.7 (7)
24	97.4 (8)	158.8 (8)

was of the same magnitude as that reported by Long and his associates (9) for mice given injections of cortin and allowed ration *ad libitum*.

At the same time that the mice were sacrificed for the glycogen determination, blood was obtained for the analysis of sugar. There was a daily variation with the feeding habits of the mice in both groups, yet the well fed mice showed a consistently higher level of blood sugar (table 2). This finding is in agreement with the earlier observation of Rusch, Johnson and Kline (10).

It is well known that the administration of certain adrenal cortical steroids to a fasting mouse will prevent the expected depletion of liver glycogen reserves (9). This principle was the basis for an experiment to test the endogenous production of cortical hormones by mice on restricted and *ad libitum* feeding habits. The fast was

begun by removing the food containers from the cages of both groups of mice at 9:00 A.M., 24 hours after the last feeding time, and accordingly this point is represented as zero time in the presentation of the data (fig. 2). It also follows that the glycogen values at this time are the same as at 9:00 A.M. in the experiment described in the preceding paragraphs. In the case of mice on the *ad libitum* regimen, the level of liver glycogen rapidly fell to values below 100 mg. per cent and it remained at this low level for 60 hours when it increased to about 700 mg. per cent, an observation similar to that made by Mirski and his associates (11). This probably indicated stimulation of adrenal cortical activity after the stress of an acute starvation. In contrast, the content of glycogen in the liver of restricted mice never decreased below the zero time level of 700 mg. per cent and was indicative of a high level of cortical activity prior to the period of prolonged fast. The length of the experiment was determined by the survival time of the mice in each group and the mice with the greater reserves lived the longer.

A more refined test of endogenous cortical activity was devised by an adaptation of the adrenal cortical assay method of Venning, Kazmin and Bell (12). They found

TABLE 3. EFFECT OF CALORIC INTAKE AND OF A GLUCOSE SUPPLEMENT UPON THE DEPOSITION OF LIVER GLYCOGEN (MG. OF GLYCOGEN/100 GM. LIVER)[1]

AD LIBITUM CONTROL	AD LIBITUM PLUS GLUCOSE	RESTRICTED CONTROL	RESTRICTED PLUS GLUCOSE
30	62	623	2250
32	68	888	2385
36	214	892	2420
64	493	1320	2450
—	—	—	—
41 av.	209 av.	931 av.	2376 av.

[1] Four mice in each group.

that the injection of small amounts of glucose such that there was no increase in the amount of liver glycogen in adrenalectomized mice increased several fold the sensitivity of the Reinecke-Kendall (13) method for the assay of cortin preparations. In the present study, the effect of small amounts of glucose on the level of liver glycogen was determined in fasted Rockland mice previously adapted to the two diets. Two intraperitoneal injections of 0.3 cc. of a 5 per cent glucose solution were given 32 and 33 hours after the last feeding time (corresponding to 8 and 9 hours after zero time in fig. 2). A total of 30 mg. of glucose was given to each mouse. The content of glycogen in the liver of both the glucose injected and control mice was determined three hours after the second injection and the results of a typical experiment are shown in table 3. The control mice did not receive glucose and the glycogen values are comparable to the 12-hour values shown in figure 2. It is noted that the administration of the glucose increased the glycogen in the liver to an average of 2376 mg. per cent in the restricted mice, but had a very minor effect on the livers of mice accustomed to a diet of abundant calories. Since the average weight of the livers is 1 gm. in the mice on the restricted diets and 1.5 gm. for those on the *ad libitum* ration, it can be seen that about 48 per cent of the total glucose administered was converted to glycogen in the underfed mice as compared to only 8 per cent in the fully fed animals.

Adrenal and Thymus Weights. In a typical experiment, 12 male mice of the ABC strain 2¼ months old were restricted to six calories daily for five weeks and 8 mice were allowed ration *ad libitum* (between 10 and 11 cal. per mouse daily). The average weight of the mice at five weeks, the average weight of the glands, and the weight of the glands expressed as mg/gm. of body weight are shown in table 4. There was no difference in the absolute weight of the adrenals, but the adrenal weight relative to the body weight was more than half again as large. These findings agree with those reported by Quimby (14) for underfed young male rats but differ from those of Mulinos and Pomerantz (15). The latter investigators found an increase in adrenal weight in rats on complete starvation but a decrease in the weight of the adrenal when the animals were kept on a diet restricted by 50 per cent. The present

TABLE 4. BODY WEIGHTS AND WEIGHTS OF ADRENAL AND THYMUS GLANDS AFTER 35 DAYS OF RESTRICTED AND AD LIBITUM CALORIC INTAKE

	RESTRICTED	AD LIBITUM
No. of mice	12	8
Av. body wt.	16.7 gm.	26.0 gm.
Av. adrenal wt.	2.38 mg.	2.36 mg.
Wt. ratio, adrenal/body	0.144 (0.022)[1]	0.091 (0.010)[1]
Av. thymus wt.	3.86 mg.	25.2 mg.
Wt. ratio, thymus/body	0.236 (0.155)[1]	0.972 (0.168)[1]

[1] Standard deviation $\left(S = \sqrt{\dfrac{\Sigma x^2}{(N-1)}} \right)$.

TABLE 5. EFFECT OF CALORIC RESTRICTION ON THE WEIGHT OF THE OVARIES AND THE UTERUS

	RESTRICTED	AD LIBITUM
No. of mice	6	8
Av. body wt.	20.7 gm.	28.6 gm.
Av. ovarian wt.	8.39 mg.	20.5 mg.
Wt. ratio, ovarian/body	0.408	0.717
range	(0.281-0.606)	(0.452-1.06)
Av. uterine wt.	18.5 mg.	86.8 mg.
Wt. ratio, uterine/body	0.903	3.07
range	(0.361-1.85)	(1.23-5.47)

experiment also showed a striking involution of the thymus gland of restricted mice which amounted to a factor of seven on the actual weight basis and of four on the relative basis (table 4).

Lymphocyte Counts. The average total leukocyte and lymphocyte count of 6 ABC control mice fed *ad libitum* were 10,300 and 8000 respectively, and of 16 mice restricted in calories, 5700 and 1900. The decreased lymphocyte count was largely responsible for the lower total counts found in restricted mice.

Adrenal Ascorbic Acid. The ascorbic acid content of the adrenals of the strain ABC mice on the two diets was also determined. There was no difference between the two groups. Data from a typical experiment showed that this value averaged 234 mg/100 gm. of adrenal (s.d. 9.4) for 8 restricted mice and 237 mg/100 gm. (s.d. 17.6) for 8 mice on the full fed diet.

Ovarian and Uterine Weights. For this experiment 8 young adult female mice

of the Rockland strain were fed *ad libitum* and 6 were restricted in calories to 60 per cent of the other group. After 53 days the ovarian and uterine weights were determined and the ratio of these weights to body weights was calculated. The data, presented in table 5, showed that average weight and the weight ratio of these two organs was reduced by 40 per cent or more in caloric restriction.

As evidenced by the vaginal smear technic, estrus ceased in all mice restricted to six calories daily, but was normal in all mice allowed 10 or more calories of the control ration. In order to determine whether the anestrus may have resulted from a decreased production of estrogens or to an inability of the tissues to respond to the hormone, the mice on the restricted diet were injected subcutaneously with 0.08 μg. of estradiol benzoate. Estrus resulted in all the mice which proved that the vaginal epithelium was still capable of responding to this stimulus. This is a confirmation of an earlier short-term experiment by Mulinos *et al.* (16) in which estrus was restored in rats subjected to complete starvation following the injection of either estradiol monobenzoate or gonadotropic hormone (Follutein).

DISCUSSION

Direct methods for the measurement of pituitary-adrenal-cortical activity have yet to be developed for so small an animal as the mouse. However, there are several measurable physiological criteria that indicate the state of this system. An involution of the thymus gland (17), a decrease in the lymphocyte count (18), an increase in the weight of the adrenal gland (19, 20) and a decrease in its content of ascorbic acid (21), and an increase in gluconeogenesis (9) are some of the manifestations of an intensified adrenal cortical activity. The finding of these changes in the mice restricted in calories strongly support the conclusion of a stimulated cortex in this condition. The one exception was the lack of a measurable change in the ascorbic acid content of the adrenal. Apparently the decrease in ascorbic acid is manifest only after conditions of acute stress (22), since similar changes have not been reported during the mild chronic stress described as Type II by Sayers *et al.* (21).

The experiments in which liver glycogen was determined in fasting mice previously adapted to the two diets and given small glucose supplements are particularly significant. The fast eliminated the cyclic effect of food consumption on the amount of glycogen (fig. 1) and permitted a greater spread in the glycogen content following the administration of low levels of glucose to the two groups. By standardizing the response in liver glycogen to that obtained with known amounts of cortical steroids, this technique is being developed for the assay of the endogenous production of hormones of the adrenal cortex.

The high content of glycogen in the livers of mice on a low caloric intake probably arises in part from non-carbohydrate sources such as amino acids and protein. These adrenal controlled glycogen precursors can be designated as the building block reserve. In contrast, the well fed mouse has a rate of cortical steroid secretion which apparently causes the conversion of only negligible amounts of this reserve to carbohydrate, as is shown by the lower content of liver glycogen. Perhaps this regulation by the adrenal explains the inhibiting effect of caloric restriction on tumor formation. In the restricted animal all available nutrients may well be required for the maintenance

of life, but in the well fed animal there is sufficient energy to satisfy the critical requirements and still allow a reserve of building blocks that could be readily available to respond to carcinogenic stimuli with the ultimate appearance of a tumor. These conditions apply only during the early stages of tumor development and have little effect after the establishment of an independent blood supply to the neoplasm.

The results of this experiment appear to be the first demonstration of an activated pituitary-adrenocortical mechanism in mice on prolonged caloric restriction. In contrast, the decreased ovarian and uterine weights and the cessation of estrus in the restricted mice reflect a lowered secretion of the gonadotropic hormones, a conclusion also reached by Huseby and his associates (4). This variance in the elaboration of two of the hormones of the pituitary suggest that the 'general adaptation syndrome' described by Selye (23) might be operative in the mouse restricted in calories with a shift to the production of the essential adrenocorticotropin at the expense of the less critical hormones. Accordingly, the term pseudohypophysectomy used by Mulinos and Pomerantz (24) to describe the condition in chronic or complete starvation cannot be used to accurately characterize the condition of the pituitary in the mouse chronically restricted in calories.

Although mice on restricted diets were in anestrus, it is significant that they respond normally to physiological amounts of estradiol. This demonstrated that the end-organ response was not impaired and that cell proliferation was not incompatible with caloric restriction.

SUMMARY

The state of activity of the adrenal cortex and of the ovary and uterus was investigated in two groups of young adult albino mice maintained for long periods on diets varying as to caloric value. One group was allowed a diet *ad libitum* while the other group was restricted in calories to 60 per cent of the other.

The mice on the restricted diet were in anestrus, but they responded normally to physiological amounts of estradiol. Thus, cell proliferation was still compatible with caloric restriction. The study of the estrus cycle together with the determination of ovarian and uterine weights indicated a lowered ovarian hormone production in mice restricted in calories. In contrast to this, these same mice showed evidence of an increased activity of the adrenal cortex; the involution of the thymus, the decrease in lymphocytes, the increase in the ratio of the adrenal to body weight and the increase in gluconeogenesis all favored this concept. The apparent variance in the secretion of the gonadotropic and adrenocorticotropic hormones from the pituitary suggested that the 'general adaptation syndrome' might be operative in mice restricted in calories, i.e. a shift by the pituitary to the elaboration of the essential adrenocorticotropin at the expense of other less critical hormones. The relation of these findings to the inhibiting effect of caloric restriction on tumor formation is described.

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STUDY OF IN VITRO METHODS FOR THE DEMONSTRATION OF ISO-AGGLUTINATION WITH THE BLOODS OF NORMAL AND OF ILL DOGS

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IN THE course of an investigation of the source of the factor responsible for the individual specificity of normal dog plasma or serum (1) and ·the relationship of this factor to the urticaria elicited by infusions of plasma from other dogs, an additional syndrome resembling anaphylactic shock was observed occasionally (2). While all dogs were sensitive in varying degrees to the urticaria-producing factor, anaphylactic shock was not necessarily produced in each of two recipients. *In vivo* hemolysis was not demonstrable in the reacting dog. Occasionally the reaction was detected only with the aid of certain laboratory criteria, particularly leucopenia and delayed disappearance of injected bromsulphalein. The phenomenon appeared unexplainable in certain instances except on the basis of blood group incompatibility, with the inclusion of soluble agglutinogen or natural iso-agglutinins in the injected plasma.

It is generally believed that distinct iso-agglutination does not occur with normal dog blood (3) and that preliminary blood grouping in dogs is unnecessary for ordinary experimental procedures. Isohemolysis and iso-agglutination have been observed, however, following sensitization by repeated transfusions of whole blood or washed erythrocytes (4–6). Using iso-immune sera, von Dungern and Hirszfeld (7) and Brockmann (8) were able to establish the existence of two agglutinogens in dog erythrocytes, giving rise to four blood groups.

The presence of natural iso-agglutinins in dog serum was reported in 1913 by Ottenberg, Kaliski, and Friedman (4) and was later confirmed by McEnery and associates (9). The success of the agglutination test was attributed by the originators to the fact that saline solution was not added to the reacting system which consisted of one volume of defibrinated blood mixed with 19 volumes of serum. The test was made at room temperature since incubation at 37° C. resulted in hemolysis or in equivocal results. The agglutinations were scattered in such a way, however, that no definite groupings comparable to human blood groups could be determined.

Because of non-specific hemolysis, absence of agglutination, or occasional non-specific agglutination, the cell-serum test of Ottenberg proved unreliable in our hands as evidenced by transfusion reactions in previous investigations. The present report concerns the development of a dependable *in vitro* method for demonstrating iso-agglutination in dog blood. The method, which employs principles elaborated by Diamond *et al.* (10) and by Wiener (11), involves the resuspension of saline-washed dog erythrocytes in albumin solution and testing in albumin-diluted dog serum.

METHOD

Dogs. In the selection of dogs animals were purposely included in all states of nutrition and disease, including moribund dogs as well as normal healthy animals. The dogs were mature, represented both sexes, and as a rule were mongrel in type.

Equipment and Solutions. All equipment used in the study was chemically clean and was sterilized by heat. The solutions used included sterile 0.85 per cent sodium chloride prepared with redistilled water, bovine albumin solution, and salt-poor human serum albumin readjusted to a salt content of 0.9 to 1.0 per cent.

Preparation of Erythrocytes. The following procedure was used: Venous blood was withdrawn without foaming into a chilled saline-wet syringe, one volume of blood being transferred into 6 or 7 volumes of cold saline, centrifuged at 2500 r.p.m., and the supernatant fluid discarded within two minutes of collection of the blood. Two more washings were carried out similarly within four minutes. This rapidity of manipulation was employed 1) in order to keep the temperature as near 4°C. as possible during the entire procedure and 2) in order to avoid having to use anti-coagulants. The above process is really one of differential centrifugation in the cold, the platelets, white cells, and lighter erythrocytes being discarded with the supernatant fluid. The method proved to be highly effective for the preservation of dog erythrocytes. Uniformity of results required, however, that the cells be incubated for a minimum of 15 minutes at 37° to 40°C. and be washed one or more times in warm saline before suspension in albumin solution for the tests of the day.

Preparation of Serum. A portion of the blood removed for preparation of cells was transferred to a chilled saline-wet centrifuge tube kept at 4°C. and the serum separated in the cold by centrifugation within about three hours of blood collection. This procedure delayed coagulation of the blood, but it was more effective in the removal of cold auto-agglutinins in one stage than was the procedure of chilling after the clot had formed and partly retracted at room temperature. For routine testing sera were stored at 7°C. for a maximum of about one week.

On the day of testing the serum was diluted with sufficient albumin solution to give a final concentration of 10 to 15 per cent albumin in the cell-albumin-serum mixture.

Agglutination Test. The test was conveniently performed by adding one drop of cell suspension (5%) to a mixture of two drops each of serum and of 30 per cent bovine albumin solution in a glass tube (7.0 mm. inside diameter). After mixing, the tubes were centrifuged immediately for 30 seconds at 2500 r.p.m. and the cells resuspended by shaking for the purpose of detecting the presence of any cold auto-agglutinins reacting rapidly at room temperature. The tubes were then incubated for 30 minutes to one hour at 37°C. and after a second brief centrifugation the presence and degree of agglutination were determined after gentle shaking of the warm tubes. Readings were also made at room temperature and after refrigeration at 7°C. over night.

In performing the tests each serum was tested by parallel determinations with autogenous cells and the cells of 5 to 10 other dogs. As the work progressed, the tests were set up by a schedule which permitted each new serum and cell suspension to be tested against the bloods of previously studied dogs, so that agglutinable and

non-agglutinable cells and agglutinating and non-agglutinating sera could be included in the tests of the day to check stability of the cell suspensions and specificity of the agglutinations.

<div align="center">RESULTS</div>

In vitro studies of compatibility of dog blood were made with the erythrocytes of 40 donors and the sera of 36 of these animals. The red cells of 19 dogs were tested with the sera of 25 to 36 dogs; 10 were tested with the sera of 15 to 25 dogs, and 10 were tested with 6 to 15 sera of appropriate type. In addition, multiple cross matching tests were made among 20 of the dogs during an interval of two to nine months. The study involved 1416 individual tests with albumin as a diluent.

Of 40 dogs, 20 had erythrocytes which contained specific agglutinable factors. The agglutinable cells were agglutinated by the sera of dogs having erythrocytes which lacked a specific factor agglutinable by natural dog iso-agglutinins at 32° to 37°C.

Cross Matching of Bloods of Normal Dogs. Cell-Albumin-Serum Method. Representative data obtained by the standard procedure using the cells and sera of presumably healthy dogs are illustrated in table 1. *Dogs 194* and *413* had received intravenous infusions of pooled or unpooled dog serum three or more months prior to this study, and *dogs 696, 628,* and *609* had received sera of other dogs by intradermal injection. The results of the tests made with the sera of these animals appeared comparable to results obtained with the sera of other animals and are included in table 1.

On the basis of cross matching among the normal animals, the symbols X, Y, and Z have been used to designate the probable groups into which the bloods could be placed. The blood of one dog (*dog 66*) contained no iso-agglutinins, and the cells gave weak atypical agglutinations which were poorly sustained at 37°C.

Other Agglutination Tests. In 97 tests packed washed red cells were added to undiluted serum. Of 36 anticipated positive reactions, seven results were negative or equivocal at 20° to 24°C. Strong rouleaux formation often complicated the interpretation of weak or negative reactions. Attempts to incubate the mixtures at 37°C. for even 5 to 15 minutes led to non-specific hemolysis in most instances. In general, cells of group Y were agglutinated while the cells of group Z gave poor agglutinations or equivocal tests.

The test using saline solution as a diluent was inadequate in confirming positive results obtained with the other methods. In 42 tests made at room temperature, four positive tests were obtained in 16 anticipated positive reactions. Non-specific hemolysis occurred rapidly at 37°C. The positive reactions were obtained with the cells of group Y, and no false positive agglutinations occurred.

Cross Matching of Bloods of Ill Dogs with the Bloods of Normal or of Ill Dogs. Blood samples were obtained from 11 chronically or acutely ill animals, and in 8 of these the bloods were obtained only during illness.

The sera of sick animals gave satisfactory results when tested against known agglutinable or non-agglutinable cells of normal dogs. In the three instances where bloods were also tested prior to illness or after full recovery, the serum prepared during illness gave the results characteristic for that animal.

TABLE I. RESULTS OF CROSS-MATCHING TESTS WITH BLOODS OF NORMAL DOGS[1]

RED CELLS			X SERUM								Y SERUM							Z SERUM			?
Blood group	Dog No.		225	354	609	328	231	353	350	270	628	194	219	413	845	352	169	696	258	244	66
X	225		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	354		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	609		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	328		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	231		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	353		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	350		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	270		o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Y	628		+++	+++	+++±	++	+++±	+++	+++±	o	o	o	o	o	o	o	o	o	o	o	o
	194		+++	+++	+++	+	++±	++±	++±	o	o	o	o	o	o	o	o	o	o	o	o
	219		+++	+++	++++	+++	+	+++	++±	o	o	o	o	o	o	o	o	o	o	o	o
	413		++++	++++	++++	+++±	+++±	++	+++	+	o	o	o	o	o	o	o	o	o	o	o
	845		++++	+++	++++	+++±	+++±	++	+++	+	o	o	o	o	o	o	o	o	o	o	o
	352		++++	+++±	++++	+++±	++++	++	+++	+	o	o	o	o	o	o	o	o	o	o	o
	169		+++	++++	+++±	++++	+++	++	++±	+	o	o	o	o	o	o	o	o	o	o	o
Z	696		+++	+++	++++	+	+++±	+++	+++	+++±	o	o	o	++	+++	±[2]	++	o	o	o	o
	258		+++	+++	+++±	+	+++±	±[2]	+++	+++±	o	o	o	+	±[2]	±[2]	+	o	o	o	o
	244		+++	+++	+++±	+	+++±	+++	+++	+++±	o	o	o	±[2]	±[2]	±[2]	++	o	o	o	o
Undetermined	66		±	o	±	±	±	o	o	o	o	o	o	o	o	o	o	o	o	o	o

[1] The strength of the reactions is indicated by crosses, for instance: Positive: ++++—one large clump, complete agglutination; +—clumps just visible to the naked eye; ±—clumps visible with hand lens. Negative: o—macroscopically homogeneous suspension. The data presented were obtained with varying final serum titers in the range of 1:2 to 1:4.

[2] Agglutination absent at irregular intervals.

Although sera free of non-specific agglutinins were fairly easy to prepare from the bloods of ill dogs, the preparation of cells free of a factor causing agglutination of cells in albumin solution was frequently tedious and time consuming. In general, smooth cell-albumin suspensions could be prepared after the cells had been incubated and thoroughly washed at 43°C. In two instances the cells required washing at 50°C., and satisfactory tests were obtained with the cells so treated, one dog having agglutinable cells and the other non-agglutinable cells by specific reactions. Two typical experiments are illustrated in table 2.

Miscellaneous Observations. Inactivation of dog serum (56°C. for 30 min.) frequently intensified its hemolytic action against autogenous or homologous cells and also weakened specific agglutinations. Although dog serum stored for one month may give no non-specific reactions, occasional weak agglutinations at room

TABLE 2.   RESULTS OF CROSS-MATCHING TESTS WITH BLOODS OF ILL DOGS AND NORMAL DOGS

BLOOD GROUPING RESULTS BY PREVIOUS TESTS	SERUM	CELLS OF ILL DOGS				CELLS OF NORMAL DOGS					
		Temp. of cell washing				Cells washed at 43°C.			Cells washed at 37°C.		
		37°C.	43°C.	37°C.	43°C.						
	Dog No.	270	270	247	247	219	628	244	66	609	328
X	270	+	o	++++	++++	++++	+++±	+++±	o	o	o
Untested	247			+±	o	o	o	o		o	
Y	219	+±	o	+±	o	o	o	o	o	o	o
Y	628		o		o	o	o	o	o	o[1]	o[1]
Undetermined	66	+±	o		o	o	o	o	o	o	o
X	609	+±	o		+++	+++±	++++	+++±	±	o	o
X	328	+±	o		+++±	++±	+++++[1]	+[1]	o	o[1]	o

Data illustrating specific and non-specific agglutinations with the bloods of ill dogs. *Dog 270* appeared normal until following a large blood donation. *Dog 247* exhibited chronic inanition following intestinal resection.

[1] Values were obtained prior to these experiments.

temperature, especially of autogenous and homologous cells, have occurred with sera stored two to four weeks.

For routine tests cells were seldom used after more than two days' storage. However, red cells prepared by cell segregation through selective centrifugation in the cold remained free of spontaneous hemolysis in saline for 6 to 10 days at 7°C. and could usually be agglutinated at room temperature. Such cells in albumin-diluted serum, however, withstood incubation at 37°C. very poorly. The specially prepared cells suspended in albumin were unusually resistant to spontaneous hemolysis even when foamed and left at room temperature for the day or when stored at 7°C. for several weeks.

Although microscopic examinations were not made routinely on negative macroscopic tests, non-agglutinability of the cells of normal dogs in group X and non-agglutinability of cells of group Y by the sera of dogs of group Y was repeatedly confirmed by microscopic examination.

DISCUSSION

The results of this study confirm the original conclusion of Ottenberg *et al.* (4) that iso-agglutinins occur in the bloods of normal dogs and can be demonstrated in a cell-serum system even though the classical cell-saline-serum method usually gives negative results.

Because of non-specific hemolysis, frequent false negative agglutinations, or auto-agglutinations, the *in vitro* method of Ottenberg *et al.* (4) has proved unreliable in our hands. By using serum albumin solution as a medium for the suspension of specially prepared dog cells and as a diluent for serum previously exhausted of interfering auto-agglutinins active at room temperature or higher, both specificity and increased sensitivity have been obtained with dog blood in macroscopic agglutination tests.

According to current theories conglutination of red cells suspended in serum or albumin solution by an antibody which fails to give visible agglutination when the serum is diluted with saline solution signifies that an antibody of the 'univalent' or

TABLE 3

BLOOD GROUPS		AGGLUTINOGEN IN RED CELLS	ISO-AGGLUTININ IN SERUM	ISO-AGGLUTININ OF INFREQUENT OR IRREGULAR OCCURRENCE
Designation	Incidence			
	%			
X	50	B	α, α_1	
Y	40	BA_1	None	α_2
Z	7.5	BA_2	None	α_1 (not observed)
Undetermined	2.5	B (?)	None	?

'blocking' type is present (10, 11). Whether or not this explanation applies to the iso-agglutinations obtained with dog sera is unknown. That inhibition of non-specific hemolysis by albumin was an essential factor in the success of this method is suggested by the observed remarkable power of albumin (10 to 15%) to preserve specially prepared dog cells and to inhibit or delay hemolysis in cell-albumin-serum mixtures during incubation at 37°C. The observations do suggest, however, that the factor adsorbed on certain cells and giving rise to weak agglutination in albumin solution in the absence of serum may be an auto-agglutinin of the 'blocking' type.

The agglutination patterns obtained with the cell-albumin-serum method indicate that the bloods of most mongrel dogs can be placed in one of two major groups. Fifty per cent of the dogs had erythrocytes which were not specifically agglutinable, whereas their sera agglutinated all erythrocytes possessing agglutinable factors. Of the remaining dogs, all had agglutinable cells, and iso-agglutinins were absent except in 4 dogs in which the sera contained weak iso-agglutinins at irregular intervals. Some reluctance was felt in assigning any significance to these inconstant agglutinations of the cells of 3 dogs of group Z by the sera of 4 dogs of group Y. However, in a similar situation, the blood of a dog of group Z (*dog 696*) produced a non-hemolytic reaction on primary transfusion into the recipient. The recipient's response consisted of salivation, severe leukopenia, and delayed disappearance of

bromsulphalein.   This incompatibility may be analogous to the *in vivo* incompatibilities encountered occasionally in humans of groups A_1 and A_2.

According to reports in the older literature the bloods of some dogs absorb anti-A agglutinins, and all dog erythrocytes absorb the human anti-B agglutinin (3, 8). Brockmann (8) in 1911 demonstrated that the sera of some normal dogs specifically agglutinate group A cells of human blood.   Data not presented in the present report established the fact that the sera of all the dogs assigned to group X contained agglutinins strongly active against human A_1 and A_1B cells.   Although α_1 agglutinins were lacking in the sera of animals possessing agglutinable cells, the sera of some of the dogs of group Y contained weak anti-O (α_2) agglutinins (2).

If all dog erythrocytes contain an agglutinogen resembling that of human B cells and if the erythrocytes of certain dogs contain an A-like agglutinogen, it appears probable that the latter agglutinogen is responsible for the iso-agglutinations found in dog blood.   The blood groupings illustrated in table 3 are therefore suggested as being consistent with observations reported in the literature and with those described in this report.

According to this hypothesis and by analogy to the human groups, the two specific dog agglutinogens detected with iso-immune sera by von Dungern and Hirszfeld (7) and designated as A and B by them would resemble those of human A bloods.

CONCLUSIONS

1. Dog bloods contain natural iso-agglutinins.

2. The hemolysis occurring in normal dog blood is non-specific.

3. Ninety per cent of mongrel dogs can be placed in one of two major blood groups.

4. A dependable method has been developed for the demonstration of iso-agglutinations in dog blood.

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MECHANISM OF PROTEINURIA. EFFECT OF PARENTERAL BOVINE ALBUMIN INJECTIONS ON HEMOGLOBIN EXCRETION IN RATS

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DURING wartime investigations into the use of substitutes for plasma, it was found by Addis and his associates (1) that massive proteinuria could be induced in rats by the intraperitoneal injection of many proteins. The degree of proteinuria varied with the dose of protein injected and with the specific protein used. Thus, the proteins of small molecular size, such as Bence-Jones protein, appeared in the urine promptly and in large quantities. On the contrary, rat serum, with its homologous protein, required greater dosage and produced less proteinuria.

The mechanism of such induced proteinuria is of considerable interest. While the situation is artificial, from a clinical standpoint, an understanding of its mechanism might indicate directions for investigating the occurrence of proteinuria in human subjects.

Determination of the factors involved in proteinuria raises special difficulties. Such methods as those requiring protein clearance determinations are complicated by difficulties in labelling and identifying specific proteins. The absence of a simple and reliable method for determining albumin, and distinguishing it from other serum proteins, imposes a great handicap. Proteins linked to dye radicals are often toxic, so that they may not be used. Radioactive substances require techniques and facilities that have not been available to us at this time.

This investigation has been based on the use of hemoglobin as an indicator substance. A 7 to 8 per cent purified and non-toxic solution of human hemoglobin (2) was used. This protein is naturally tagged with an identifiable color and with its content of iron.

The excretion of hemoglobin was studied after intravenous injection of various doses in rats, in which a heavy proteinuria had first been induced by intraperitoneal injections of bovine albumin. Under the conditions chosen, protein excretion rates of from 1000 to 1500 mg/24 hrs. are attained during the experimental period. When it is realized that the weight of both kidneys in such a rat is little more than 1000

mg., the great degree of proteinuria may be appreciated. The results were compared with the hemoglobin excretion of rats that received intraperitoneal injections of 0.85 per cent sodium chloride solution. In this way, it was possible to study the effect of bovine albumin injections, with consequent massive proteinuria, upon the excretion of another protein molecule of similar size, but different shape: hemoglobin.

<div align="center">METHODS</div>

In this investigation, 211 rats each weighing about 150 gm. were used. They were fed a stock diet containing 17 per cent protein. On the day preceding hemoglobin injection, the experimental rats received two 16-cc. intraperitoneal injections of 6 per cent bovine albumin in 0.85 per cent sodium chloride solution, one given at 9:00 A.M. and the other at 4:30 P.M. On the next day, the rats received another such injection at 9:00 A.M., so that the total amount of albumin injected was approximately 3 gm. Just $5\frac{1}{2}$ hours later, a time selected to coincide with the peak of the proteinuria, the rats received an intravenous injection of hemoglobin, varying from 4.1 mg. to 141.0 mg. in a volume of 1.7 cc. The highest dosage represented undiluted stock hemoglobin solution. Lower dosages were prepared by diluting the stock solution with 7.0 per cent bovine albumin solution, so that the injection would introduce a relatively constant osmotic effect. The amount of albumin so injected, as is known from previous experiments (1), would not appreciably influence the control values and in any case would diminish rather than magnify the experimental difference. All injections were performed under light ether anesthesia. Control rats were treated in precisely the same way, but received intraperitoneal injections of 0.85 per cent sodium chloride solution, without added protein.

At each dosage of hemoglobin injected, a group of rats was killed two minutes after the end of injection and bled from the abdominal aorta in order to obtain the initial hemoglobin concentration in the serum. Much previous experience has shown that no hemolysis occurs under the conditions of blood collection used. Other groups were killed 30 minutes after the end of injection. In the latter groups, a final hemoglobin concentration was obtained. Kidney weight was measured in all rats killed. Urine was collected during the 30-minute waiting period for determination of hemoglobin excretion. Determinations were performed on individual specimens.

Total hemoglobin was measured by the method of Evelyn and Malloy (3). Rates of excretion have been expressed in terms of predicted kidney weight (KWP) for a rat of the observed body weight. The size of the kidney is the best means of reducing structural variations to comparable terms. The conditions of this experiment produce changes in kidney weight which will be described later, rendering it necessary to use the predicted kidney weight, which has been derived from a mass of normal data concerning rats of the same colony (4). Midpoint hemoglobin concentrations were calculated, assuming a logarithmic rate of fall in concentration during the experimental period (fig. 1).

<div align="center">RESULTS AND DISCUSSION</div>

Both the experimental and the control rats had a pronounced diuresis after the hemoglobin injection. Hemoglobin appeared in the urine as soon as two minutes after the injection was completed.

In the control rats injected with sodium chloride solution there appeared to be a threshold for the excretion of hemoglobin at a serum concentration of approximately 75 mg/100 cc. At this level, most of the urine specimens were negative to the benzidine reaction. Above this level, the increase in hemoglobin excretion bore a linear relation to the serum hemoglobin concentration (fig. 2).
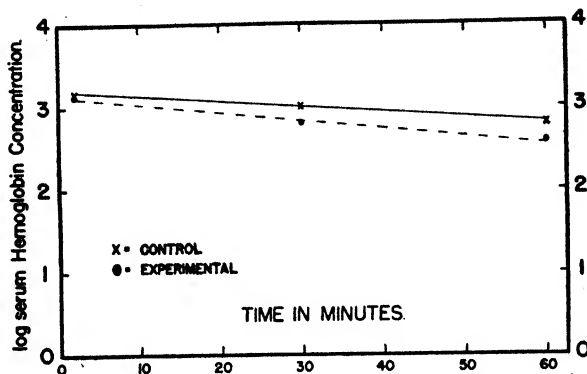
Fig. 1. Fall in serum hemoglobin concentration after single intravenous injection
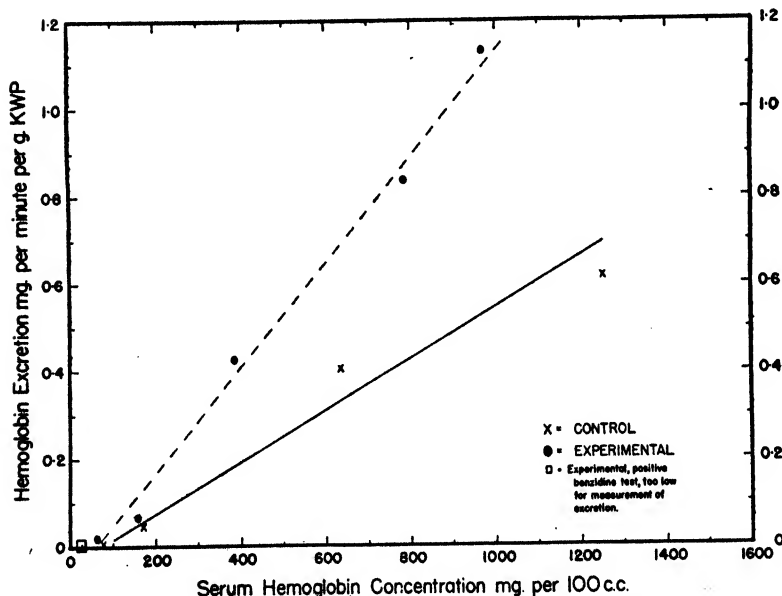
Fig. 2. Relation of hemoglobin excretion to serum concentration

In the experimental rats injected with bovine albumin no threshold could be detected at the lowest serum concentration that could be measured with accuracy, namely, 25 mg/100 cc. Although we were not able to measure the excretion quantitatively at this point, benzidine reactions of the urine were strongly positive in all specimens. Above this level, there was again found a linear relation between the excretion of hemoglobin and the serum concentration. However, the hemoglobin

excretion was much higher than in the control rats, being approximately double at comparable serum hemoglobin concentrations (table 1).

In the dog, Monke and Yuile (5) found a serum concentration threshold at about 100 mg/100 cc., below which hemoglobin did not appear in the urine. Our figure of about 75 mg/100 cc. in the control rats corresponds fairly well with their earlier finding. They estimated the ratio of hemoglobin clearance to glomerular filtration rate in the dog to be 0.03, while in our control animals, using data from other experiments on renal clearances, the ratio was estimated roughly to be 0.04.

Control animals show a slight rise in kidney weight (6%), under the experimental conditions, over the normal weights for rats of the same size on stock diet. The experimental animals, however, show a 22 per cent increase in kidney weight. This change in weight could be due to work hypertrophy and hyperplasia, with an

TABLE 1.   EFFECT OF BOVINE ALBUMIN INJECTIONS ON HEMOGLOBIN EXCRETION, AT VARIOUS SERUM HEMOGLOBIN CONCENTRATIONS

SERUM HGB. CONC. AT 2 MIN.	SERUM HGB. CONC. AT 30 MIN.	CALCULATED[1] SERUM HGB. MIDPOINT CONC.	HEMOGLOBIN EXCRETION	HEMOGLOBIN CLEARANCE	BENZIDINE REACTION
Control					
mg/100 cc.	*mg/100 cc.*	*mg/100 cc.*	*mg/min/G.KWP*	*cc/min/G.KWP*	
83	70	77	0.0073	0.00947	Most neg.
193	147	170	0.0480	0.0282	
791	496	636	0.409	0.0643	
1492	1025	1253	0.619	0.0494	
Experimental					
27	23	25			All strong. pos.
69	61	65	0.0177	0.0272	
186	131	158	0.0669	0.0424	
528	269	386	0.428	0.111	
1066	551	785	0.840	0.107	
1403	634	970	1.134	0.117	

[1] Calculated midpoint assumes that rate of fall in concentration is a log. function of time.

increase in the amount of functioning renal tissue, or it might be due to dilatation of the tubules with fluid and protein, resulting in a non-functional weight increase. Histological studies were performed[2] to distinguish such changes. The tubular lumina of the experimental group contained dense hyaline coagula, representing protein in high concentration. Such coagula, unlike urine, do not escape when the kidney is removed, decapsulated, sectioned, blotted and weighed, and this probably accounts for the weight increase in the kidneys of the experimental group. There was no significant difference in the number of intracellular hemoglobin droplets in the proximal convoluted tubule cells of the control and experimental groups. The urea excretion and urea concentrations, measured in serum and urine after three

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[2] Histological examinations were performed by Dr. Lelland J. Rather, Stanford Univ. School of Medicine.

injections of bovine albumin, were no greater than after three injections of sodium chloride solution, so that there did not appear to be any basis for expecting work hypertrophy. Therefore, results were expressed in terms of kidney weight predicted for rats of the same size. However, the differences in hemoglobin excretion between control and experimental groups were so large that they would have appeared significant even though the observed kidney weights had been used.

In view of the threshold shown by normal animals to the excretion of hemoglobin in the urine, it seems likely that, as Yuile has suggested, a given percentage of the hemoglobin molecules presented to the glomerular membrane pass through, and, until the capacity of a reabsorption mechanism is exceeded, do not appear in the urine. Once saturation is achieved, the excretion of hemoglobin becomes a linear function of the serum concentration.

The injections of bovine albumin, by creating an experimental proteinuria, might be expected to saturate the reabsorption mechanism in a non-specific manner. If this were so, the threshold for hemoglobinuria would be lowered, as was observed. However, if that were all, it would then be expected that the line relating excretion to serum concentration in the experimental rats would be parallel to and above the control line. Since the line based on the experimental observations has a much steeper slope than the control line (fig. 2), there must have been an increase in the amount of hemoglobin passing the glomerular membrane.

An increase in the glomerular filtration rate might allow passage of additional quantities of hemoglobin, with no alteration in the glomerular permeability. We have obtained preliminary results which seem to indicate that the difference between inulin clearances determined for the experimental rats and the controls are neither of magnitude nor of direction that would explain the difference in hemoglobin excretion. It seems probable, therefore, that the increase in excretion must be associated with an increase in the glomerular permeability to hemoglobin. Further experiments are in progress, which will attempt to isolate the functions of filtration and reabsorption of hemoglobin.

SUMMARY

Intraperitoneal injections of bovine albumin double the excretion of intravenously administered hemoglobin by the rat kidney, at comparable serum hemoglobin concentrations. Under these conditions, the serum concentration threshold at which hemoglobin appears in the urine is lowered from about 75 mg/100 cc. to less than 25 mg/100 cc. It is suggested that the observed results can be explained by the saturation of a protein reabsorption mechanism and an increase in glomerular permeability to hemoglobin.

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SALYRGAN AND RENAL TUBULAR SECRETION OF PARA-AMINOHIPPURATE IN THE DOG AND MAN[1]

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MARKED depression of the tubular secretion of diodrast and para-amino-hippurate (PAH) following the injection of salyrgan (mersalyl)[2] in human subjects has been reported by Brun, Hilden, and Raaschou (1). An earlier observation in this laboratory had indicated that this function in the dog was not impaired during mercurial diuresis. The discrepancy seemed to warrant further investigation.

METHODS

Animal experiments were performed on trained, unanesthetized female dogs. Observations in man were obtained using patients with no evidence of renal disease.

Inulin in patients, creatinine in dogs and PAH in both were administered by continuous intravenous infusion. All urine collections were made with an indwelling catheter and each clearance period was terminated by washing the bladder with distilled water. Heparinized venous blood samples were obtained at the midpoint of each clearance period. The periods were of 10 to 20 minutes duration depending on the urine flow.

Creatinine was determined in tungstic acid filtrates of plasma and in diluted urine by a modification of the Folin method (2). Inulin was determined in zinc filtrates of plasma and in diluted urine by the method of Harrison (3). Glucose was removed from plasma by treatment with yeast before precipitation. PAH was determined in trichloroacetic acid filtrates of plasma and in diluted urine by the method of Bratton and Marshall (4). Sodium was determined in urine and plasma by internal standard flame photometry (5) with an error of less than one per cent. Chloride was determined by a modified Volhard titration (6).

The secreted PAH (T_{PAH}) was calculated as the difference between excreted and filtered PAH. In the calculation of the filtered PAH, 83 per cent of the plasma PAH was assumed to be free in the plasma water (7). Since marked disturbances of plasma proteins were not present in the experimental subjects and since interest centered upon changes in transfer capacity (Tm_{PAH}), rather than on its absolute value, the use of this empirical factor in both dog and man does not significantly influence the

results.   Loads presented to the tubule for secretion were calculated on the assumption of a filtrate fraction of 0.20 in man and 0.30 in the dog.

The pattern of all experiments was similar.   Each was initiated with a priming dose of PAH and inulin (or creatinine).   After 20 to 40 minutes for equilibration two or three control urine collection periods were obtained.   A dose of salyrgan, 2 ml. in patients, one ml. in dogs (or 4 to 6 mg. HgCl₂ in some dog experiments) was then administered intravenously.   An interval of 30 to 45 minutes was allowed for the mercurial to exert its effect before the three or more post-mercurial urine collection

TABLE 1.   EFFECT OF SALYRGAN ON $T_{M_{PAH}}$ IN THE DOG (EACH FIGURE IS THE AVERAGE OF 3 CLEARANCE PERIODS)

DOG	BEFORE SALYRGAN		AFTER SALYRGAN			AVERAGE RATIO, LOAD/T
	T_{PAH}	Sodium excretion	T_{PAH}	ΔT_{PAH}	Sodium excretion	
	mg./min.	*μeq/min.*	*mg./min.*	%	*μeq/min.*	
B	30.1	93	32.1	+6.7	415	1.8
E	12.8	162	12.5	−2.3	822	5.5
F	17.4	136	17.5	+0.6	863	2.0
M	12.4	260	10.7	−13.7	1652[1]	5.0

[1] Within 30 min. of the injection of BAL (3 mg/kilo) the sodium excretion and urine flow were reduced below control levels without change in the $T_{M_{PAH}}$.

TABLE 2.   EFFECT OF SALYRGAN ON $T_{M_{PAH}}$ IN MAN (EACH FIGURE IS THE AVERAGE OF 2 TO 3 CLEARANCE PERIODS)

PATIENT	BEFORE SALYRGAN				AFTER SALYRGAN				
	T_{PAH}	Load/T	Chloride excretion	Sodium excretion	T_{PAH}	Load/T	ΔT_{PAH}	Chloride excretion	Sodium excretion
	mg./min.		*μeq/min.*	*μeq/min.*	*mg./min.*		*per cent*	*μeq/min.*	*μeq/min.*
H	106	1.0	149	692	34.5	5.0	−67	382	867
L	67.5	1.0	12	286	16.4	6.2	−76	258	368
M	71.5	1.6	204	617	13.7	11.0	−81	297	532
P	71.0	1.9	233	663	17.5	13.0	−75	475	890
T	113	1.5	47	409	19.7	18.0	−83	1415	1292

periods were obtained.   In some experiments a dose of 2,3 dimercaptopropanol (BAL) was then administered intramuscularly and several additional urines collected.

RESULTS

The effect of salyrgan on $T_{M_{PAH}}$ was observed in four experiments on 4 dogs. The results are summarized in table 1.   In no instance was there any significant change in the $T_{M_{PAH}}$, despite the striking increase in sodium excretion and the usual diuresis.

A sharp difference is apparent in man.   The results of experiments in 5 normal subjects are presented in table 2.   In each experiment there was a marked depression of the secretion of PAH, ranging from 67 to 83 per cent.   In several experiments (*subjects H and L*) the load presented to the tubules for secretion in the presalyrgan

periods was probably too small to insure saturation of the mechanism.   The Tм in these patients was probably higher than that observed and the depression of the secretory capacity greater than indicated by the figures obtained.

The protocol of the experiment on *patient T* is presented in detail in table 3. The injection of salyrgan depressed the $T_{M_{PAH}}$ by about 80 per cent.   Following the administration of 5 mg/kilo of BAL, the effect of the diuretic on electrolyte excretion

TABLE 3.   PROTOCOL OF EXPERIMENT ON PATIENT T SHOWING EFFECT OF SALYRGAN AND OF BAL ON TUBULAR SECRETION OF PAH

TIME	URINE FLOW	INULIN CLEARANCE	SODIUM EXCRETION	CHLORIDE EXCRETION	PLASMA PAH	T_{PAH}
min.	*ml/min.*	*ml/min.*	*μeq/min.*	*μeq/min.*	*mg. %*	
0	Complete injection of priming inulin 3.75 gm., PAH 6 gm.					
1	Start infusion of inulin 0.9%, PAH 3.2% in normal saline at 5 ml/min.					
25–46	2.85	147	424	61	27.7	109
46–69	2.86	151	420	50	24.8	120
69–92	2.42[1]	132	382	31	24.0	110
93	Salyrgan 2 ml. i.v.					
139–155	4.16	124	561	524	50.7	10.6
155–164	12.32	146	1755	1940	56.1	22.0
166	BAL 5 mg./kilo i.m.					
164–176	10.88	115	1560	1780	60.3	25.9
176–195	3.46	136	576	40	54.5	107
195–215	2.78[1]	112	461	31	50.1	98

[1] Values of all excretion rates in these periods are probably low because of incomplete urine collection.   The increases required to bring the inulin excretion up to the av. of the remaining periods are 14 and 12% respectively.

TABLE 4.   EFFECT OF $HgCl_2$ ON $T_{M_{PAH}}$ IN THE DOG

DOG	BEFORE HgCl₂		AFTER HgCl₂			AFTER BAL		AVERAGE RATIO, LOAD/T
	T_{PAH}	Sodium excretion	T_{PAH}	ΔT_{PAH}	Sodium excretion	T_{PAH}	Sodium excretion	
	mg/min.	*μeq/min.*	*mg/min.*	*%*	*μeq/min.*	*mg/min.*	*μeq/min.*	
B	13.0	157	14.7	+13.1	384	12.8	294	6
E	17.4	272	18.3	+5.2	403	18.0	186	2
L	11.5	445	11.9	+3.5	733	13.5	483	11
M	10.9	31	9.3	−14.7	491	9.6	244	6

was very rapidly dissipated and the $T_{M_{PAH}}$ was restored almost to the control value. In two other experiments, smaller doses (up to 2.5 mg/kilo) of BAL did not have any definite effect on either electrolyte excretion or $T_{M_{PAH}}$.   It should be noted that the control sodium excretion in these experiments is high because of the cation required to cover the PAH.   The effect of salyrgan is to reduce the PAH excretion sharply and, with this, to reduce the cation excretion necessitated by the excretion of PAH. For this reason, the changes in chloride excretion are more striking than those in sodium and the chloride has been included in the summary table 2 as an indication of the diuretic effect of the salyrgan.

It seemed important to determine whether the difference between man and dog, in the response of the Tm_{PAH} to salyrgan, was attributable to differences in the susceptibility of the transfer mechanism to inhibition by mercury or to differences in the handling of the organic salyrgan molecule. Experiments were therefore performed on 4 dogs in which small doses of mercuric chloride were injected intravenously. These doses were sufficient to produce diuresis without, presumably, inducing severe renal damage and were of the same order of magnitude as those used in man by Sollman, Schreiber, and Cole (8) in a study of the diuretic effect of a number of mercury compounds. The effects on sodium excretion and on the Tm_{PAH} are presented in table 4. The diuretic effect of $HgCl_2$, although definite, was less striking in these experiments than that of salyrgan. As in the salyrgan experiments, there was no appreciable depression of the Tm_{PAH}. Injection of a single dose of approximately 5 mg/kilo of BAL depressed the excretion of sodium and chloride towards the control values. The interruption of the diuresis was not, however, as dramatic as when the diuresis had been induced with salyrgan.

The difference between control Tm values observed in 2 of the dogs, B and E, which are the same in tables 1 and 4, warrants comment. The control values of Tm_{PAH} in *dog B* indicate a fall of 57 per cent between the first and second experiments, an interval of two months. On the other hand, the Tm_{PAH} in *dog E* rose 36 per cent in the five-month interval between the two experiments. No ready explanation for these changes is apparent. Stability from month to month of the transfer capacity for PAH in the dog has, however, never been established.

DISCUSSION

The fact that the renal tubular capacity to transfer PAH in man is markedly depressed by salyrgan while in the dog it is unaffected indicates a fundamental difference in the transfer mechanism. It had long been postulated that the action of the organic mercurial diuretics is due to the liberation of small amounts of mercury ion from the organic complex (8). This is supported by the effect of BAL in completely reversing the effects of the organic mercurials on both electrolyte reabsorption and PAH secretion. Failure to depress the Tm_{PAH} in the dog does not seem attributable to a difference in the handling of the salyrgan itself. In the dog, the effect of salyrgan on electrolyte reabsorption does not differ from that observed in man. Its reversal by BAL is rapid and complete (9). Furthermore, the diuretic effect can be produced by the injection of ionic mercury without affecting the transfer of PAH.

One point of difference between dog and man in the transfer mechanism for PAH has previously been noted, i.e. the same mechanism appears to be involved in the secretion of creatinine in man (10), whereas no secretion of creatinine occurs in the dog. This may not be germane to the difference in response to mercury since salyrgan has been reported to depress the diodrast Tm of the rat (11), a species in which creatinine secretion does not occur (12).

The data reported re-emphasize the need for caution in applying information concerning tubular transport mechanisms in one species to another.

SUMMARY

The Tm_{PAH} of man is markedly depressed by salyrgan. This depression, as well as that of electrolyte reabsorption, is reversed by BAL. Salyrgan and small doses of $HgCl_2$ do not depress the Tm_{PAH} in the dog, indicating a significant difference in the tubular mechanism for transferring PAH.

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RENAL GLUTAMINASE[1]

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THE presence of significant amounts of glutamine in various animal tissues has been established (1, 2). Studies in recent years with labelled ammonia-nitrogen (3) have shown that glutamine accepts as well as contributes ammonia, providing an important means for the detoxification, storage and transportation of this base. It has also been learned that the introduction of ammonia into glutamic acid as well as the reverse process, the hydrolytic liberation of ammonia from glutamine, is catalyzed by the enzyme glutaminase (4). The hydrolytic action of glutaminase is dominant in the kidney while in other tissues the synthetic action of the enzyme seems to prevail (4, 5). It is not known whether this difference is based on different types of glutaminase or whether a shift in the equilibrium of the enzymatic reaction is caused by special activators or inhibitors operating in different tissues. The formation of urinary ammonia and its manifold increase in acidosis has been explained by glutamine hydrolysis (6). The suggestion that the stimulus for increased ammonia formation in acidosis might be related to the lowering of the pH of the plasma or the decline of its bicarbonate concentration (7) should be enlarged to include formation or mobilization of specific activators for renal glutaminase. The question arises whether under pathological conditions the equilibrium of glutaminase action in other tissues might change in the direction of hydrolysis. Were this to occur, the ammonia formed might well prove toxic at its site of formation. Studies were initiated to explore this possibility and also the presence of activators and inhibitors of hydrolysis or synthesis of glutamine. The report that follows is concerned with studies on kidney glutaminase and especially with the activation of the enzyme by phosphate ions and beta-hydroxybutyric acid.

MATERIALS AND METHODS

Freshly prepared tissue slices were used as enzyme preparation of glutaminase. Immediately following extirpation of a dog or cat kidney, the organ was speedily frozen by aid of solid CO_2 and kept in a deep freezer until used. For each determination the frozen kidney was placed on a small block of solid CO_2, very thin slices were shaved from the cortex with a razor blade, rapidly weighed and placed in a test tube containing 3 cc. of buffer and either 2 cc. of saline (blank) or 2 cc. of freshly prepared glutamine solution, containing 1 mg. of glutamine per cc.[3]

After incubating the tubes in a water bath at 37.5°C. for varying lengths of time glutaminase action was stopped by lowering the *p*H to 5.0 through the addition of 1 cc. of 1 N sulfuric acid.

After deproteinization by addition of 1 cc. of 10 per cent sodium tungstate solution and 1 cc. of $\frac{2}{3}$ N sulfuric acid, the tubes were centrifuged and an aliquot of the protein-free supernatant, made up to 5 cc. with distilled water, was placed in an Evelyn colorimeter. Five cc. of Nessler's solution prepared according to Folin-Wu were added and the content of the tubes mixed. The tubes then were read as soon as possible with a 440 mμ filter.

The rapid development of turbidity in many tubes, particularly in the glutamine-free blanks, was a vexing problem, necessitating both the use of diluted aliquots and rapid reading. To test the accuracy of the values, direct Nesslerization was checked by both the permutit and the aeration method. In both these procedures, the initial steps for the determination of ammonia were identical including the precipitation of the proteins and centrifugation mentioned above. Then 1 gram of permutit was added to an aliquot of the supernatant, the tube shaken for 30 seconds and the permutit centrifuged off. The ammonia was eluted from the permutit with 10 cc. of $\frac{1}{10}$ N HCl solution in 3 per cent NaCl, and after the permutit was centrifuged off, an aliquot of the supernatant was used for Nesslerization. For the aeration method a slightly modified Van Slyke urea apparatus (9) was used. Phosphate buffers were made up of Na_2HPO_4 and KH_2PO_4 to the various *p*H's and molarities mentioned below. Veronal buffers were made up to m/20 and the *p*H was adjusted with n/10 HCl or n/10 NaOH.

To contrast tissue slice with organ extract preparations, kidney cortex was ground with sea sand or homogenized with a Ten Broeck (10) apparatus. One cc. of the homogenate, corresponding to 800 mg. of kidney cortex was diluted as indicated below. The liberated ammonia in these experiments was determined both by direct Nesslerization and with the aeration method. Pyruvic acid used in these experiments was obtained from the Eastman Kodak Company, Rochester, New York, and beta-hydroxybutyric acid, from the Paragon Testing Laboratories, Orange, New Jersey[4].

RESULTS

Ammonia Formation in Tissue Slices in the Absence of Added Glutamine. Early in the course of these studies it was observed that ammonia formation in tissue slices prepared as described above is rather small. This is a favorable contrast to the disturbing ammonia production in extracts and homogenates that require special precautions, including prolonged dialysis at 0°C. as well as addition of potassium cyanide (5). As will be shown later glutaminase activity is so much greater in tissue slices than in homogenates or extracts that a few minutes of incubation suffice. This helps still further to reduce ammonia formation in controls.

pH Optimum of Glutaminase. pH series were prepared with m/20 veronal buffer. The maximal glutaminase activity in three sets of experiments was found to be at or

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[4] Kindly supplied by Dr. A. E. Wilhelmi.

near pH 7.8. Figure 1 is representative of the three experiments and indicates the
dependency of glutaminase activity on hydrogen ion concentration (4).

Control experiments were carried out in which veronal was replaced by different
amounts of n/100 NaOH. Since the pH despite the lack of any buffer solution did not
change more than 0.2, direct comparison with the buffered solution was possible. It
was found that the glutaminase activity was uninfluenced by the addition of m/20
veronal.

Enzyme Amount. The influence on glutamine hydrolysis of different enzyme
amounts was studied in 14 series of experiments. The weights of the tissue slices
served in lieu of volume amounts. Results were rather uniform and are represented
by the following example: Liberation of NH_3-N from 2 mg. of glutamine on 10 minute
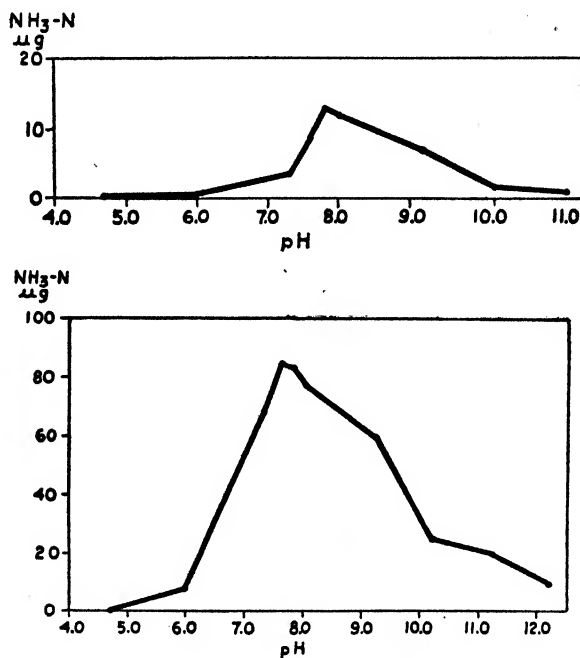
Fig. 1 (*upper*). pH CURVE OF RENAL GLUTAMINASE IN VERONAL
BUFFER.

Fig. 2 (*lower*). pH CURVE OF RENAL GLUTAMINASE IN PHOSPHATE
BUFFER.

incubation with tissue slices of 400, 150, and 80 mg. wet weight was found to be 163,
92, and 43 μg. respectively, corresponding to an hydrolysis of 85, 48, and 22 per cent.
Tissue slices of approximately 80 mg. wet weight were found to be convenient for
these experiments and henceforth were used.

Enzyme Activity in Tissue Slices as Compared to Enzyme Activity in Extracts.
The relatively low glutaminase activity of renal extracts or homogenates observed
early in these studies was one of the reasons of utilizing tissue slices. Many ex-
periments were carried out to compare quantitatively the activity of slices with
extracts and homogenates. One example follows: homogenates containing 800, 400,

and 80 mg. of cortex, liberated on 10-minute incubation 122.3, 60.8, and 13.2 μg. of NH₃-N from 2 mg. of glutamine while a cortex slice of 80 mg. liberated under identical conditions of substrate, temperature, pH and time 63.2 μg. of NH₃-N. It can be seen that weight per weight the activity of glutaminase in tissue slices is more than five times as great as that of tissue homogenates.

Substrate Amount and Incubation Time. The amounts of glutamine as well as the incubation times were varied in three sets of experiments. Table 1 is representative of one of these.

It should be mentioned that each set of experiments was carried out on slices of one individual kidney cortex. This precaution was taken after it had been observed that the glutaminase activity of kidney cortices of different dogs may vary considerably.

Activators of Glutaminase. A. PHOSPHATE. Replacement of veronal buffers with phosphate buffers left the pH optimum unchanged, i.e. near 7.8. Figure 2 represents the result of a typical experiment.

Another fact became evident in these experiments. Comparison between the curves obtained with veronal and with phosphate buffer confirmed previously reported activation of glutaminase by phosphate anions (11–13). However, the full

TABLE 1. INFLUENCE OF THE SUBSTRATE CONCENTRATION AND OF THE INCUBATION TIME ON THE ACTION OF RENAL GLUTAMINASE

GLUTA-MINE	INCU-BATION TIME	NH₃-N HYDRO-LYZED BY GLUTAM-INASE	GLUTA-MINE	INCU-BATION TIME	NH₃-N HYDRO-LYZED BY GLUTAM-INASE	GLUTA-MINE	INCU-BATION TIME	NH₃-N HYDRO-LYZED BY GLUTAM-INASE
mg.	*min.*	*μg.*	*mg.*	*min.*	*μg.*	*mg.*	*min.*	*μg.*
0.4	3	1	0.4	6	6	0.4	10	7
2.0		5	2.0		12	2.0		34.5
6.0		25	6.0		40	6.0		59

degree of this activation seems to be recognizable only by the use of the tissue slice method. The following example is representative for 6 different experiments carried out identically: liberation of NH₃-N from 2 mg. of glutamine without buffer (pH 7.8), 8.0 μg., with veronal buffer (pH 7.8) 8.5 μg. and with phosphate buffer $\left(\dfrac{m}{3}, p\text{H } 7.8\right)$ 64.0 μg.

Optimum Concentration of Phosphate for Activation of Glutaminase. Table 2 shows the influence of variation of phosphate concentration on the glutaminase activity.

As can be seen the optimal *final* concentration of phosphate was found to be between 1/4 and 1/6 molar. However even 1/30 molar final concentration activates glutaminase greatly.

B. PYRUVATE. A second acid has been tested in several tissue slice experiments. In accordance with previous observations (11–13), activation of glutaminase with pyruvate was marked. Nevertheless the action of pyruvate was significantly less than that of phosphate. When these two activators were combined they did not exhibit a cumulative effect; on the contrary, in several experiments of this kind the activation of this combination was slightly less than that of the phosphate alone.

C. BETA-HYDROXYBUTYRIC ACID. In the search for other activators of renal glutaminase, attention was directed to the circumstances existing in diabetic acidosis. On the basis of the observation that glutamine is the chief precursor of urinary ammonia (6) the large increase of the latter substance in acidosis may be referred to an increased glutamine hydrolysis. It seemed, therefore, of interest to study the influence of beta-hydroxybutyric acid on the activity of renal glutaminase. In a series of 70 experiments carried out in the course of these studies it was found that beta-

TABLE 2. INFLUENCE OF INORGANIC PHOSPHATE ON THE ACTIVITY OF RENAL GLUTAMINASE

MOLARITY OF PHOSPHATE BUFFER[1]	NH₃-N LIBERATED	WEIGHT OF SLICES
	μg.	*mg.*
1	85.1	80
1/2	97.9	
1/3	97.4	
1/5	76.6	
1/15	77.3	
1/50	13.5	
1/100	10.7	

[1] 3 cc. used in 5 cc. total volume, pH 7.8.

TABLE 3. ACTIVITY OF GLUTAMINASE IN VERONAL BUFFER, IN PHOSPHATE BUFFER AND IN PHOSPHATE BUFFER PLUS BETA-HYDROXYBUTYRIC ACID

EXP. NO.	NO BUFFER pH 7.8	VERONAL *m*/20	PHOSPHATE	PHOSPHATE + 1 MMOL. β-HYDROXY-BUTYRIC ACID
	NH₃-N μg.	*NH₃-N μg.*	*NH₃-N μg.*	*NH₃-N μg.*
1	8.5	8.0	64	141.1

TABLE 4. ACTIVATION OF RENAL GLUTAMINASE BY BETA-HYDROXYBUTYRIC ACID

EXP. NO.	PHOSPHATE BUFFER	PHOSPHATE BUFFER + 1 MMOL. β-HYDROXYBUTYRIC ACID
	NH₃-N μg.	*NH₃-N μg.*
2	50.0	90
3	18.3	62.4
4	38.1	109.3

hydroxybutyric acid activates glutaminase in veronal buffer to about the same extent as pyruvate, while in phosphate buffer it activates the enzyme to an exceedingly high degree.

Tables 3 and 4 contain the results of four representative experiments.

A fifth experiment indicating the great differences in renal glutaminase activity in veronal buffer, phosphate buffer and phosphate buffer plus beta-hydroxybutyric acid is illustrated in figure 3.

Influence of the Amount of Beta-hydroxybutyric Acid. In the six series of experiments increase of beta-hydroxybutyric acid from 0.5 to 1.0 millimole was associated

with a progressive increase in the enzyme activation. In one representative experiment liberation of NH_3-N on addition of 0.5 millimole, 1.0 millimole, and 2.0 millimole of beta-hydroxybutyric acid was found to be 66.1, 76.4, and 97.9 μg. Higher concentrations have not as yet been tested.

D. LACTIC AND ACETIC ACID. When beta-hydroxybutyric acid in veronal buffer was replaced by the same concentration of either lactic or acetic acid only insignificant activation of glutaminase was observed. When, however, these acids were used in combination with phosphate buffer there was a marked inhibition of the activating action of phosphate. Table 5 contains the results of a representative experiment of the series of 12.

Fig. 3. RENAL GLUTAMINASE ACTIVITY in veronal buffer (. . . .), in phosphate buffer (– – – – –) and in phosphate buffer plus 2 millimole of beta-hydroxybutyric acid (——).
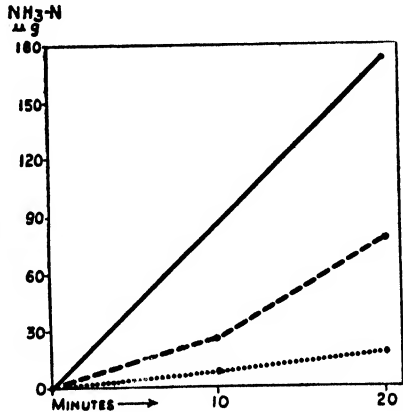
TABLE 5. ACTIVATING AND INHIBITING INFLUENCES ON RENAL GLUTAMINASE

VERONAL M/20	PHOSPHATE M/3	PHOSPHATE + β-HYDROXYBUTYRIC ACID (1 MMOL.)	M/3 PHOSPHATE + LACTIC ACID (1 MMOL.)	M/3 PHOSPHATE + ACETIC ACID (1 MMOL.)
NH_3-N μg.	NH_3-N μ .	NH_3-N μg.	NH_3-N μg.	NH_3-N μg.
10.0	42	96	24	33

DISCUSSION

The application of the tissue slice method for the determination of renal glutaminase proved to be advantageous. The enzymatic activity in slices prepared from renal cortex was significantly greater than that in extracts or homogenates of the same organ. The high activity of the enzyme permitted reduction of the incubation time to a few minutes. During this short incubation production of ammonia in the glutamine free solution (blank) was very small. The activating influence of inorganic phosphate and pyruvate, previously reported for extracts (11, 12) was also demonstrated in slices of kidney cortex. The observation that beta-hydroxybutyric acid in combination with inorganic phosphate is the strongest activator for renal glutaminase as yet observed is remarkable in the light of the conditions prevailing both in diabetic acidosis and in hyperglycemia. Increased production of betahydroxybutyric acid in diabetes is known to be associated with an increased formation of

urinary ammonia, and alimentary hyperglycemia has been recently reported to be followed by an elevated phosphatase action in renal tubules, i.e. increased liberation of inorganic phosphates (14, 15). Whether the *in vitro* results on phosphate and beta-hydroxybutyric acid suffice to explain the mechanism of increased urinary ammonia formation requires further investigation.

SUMMARY

The activity of renal glutaminase in tissue slices prepared from frozen kidney cortex is significantly greater than that in extracts or homogenates of the same organ. The stimulation of the enzymatic activity in tissue slices by pyruvate and phosphate is marked.

The greatest stimulation of renal glutaminase was obtained by a combination of phosphate and beta-hydroxybutyric acid. Possible relationship of this combination to the increased formation of urinary ammonia in diabetic acidosis is suggested.

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